

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/336533654>

# Journal of the Florida Mosquito Control Association, Vol. 66, 2019

Book · October 2019

---

CITATIONS

0

READS

615

1 author:



Rui-De Xue

Anastasia Mosquito Control District, Florida, USA

185 PUBLICATIONS 2,162 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Project

Public health insecticide evaluation [View project](#)



Project

Wolbachia pipientis [View project](#)

ISSN 1055-355X (print)  
ISSN 2638-6054 (online)

# JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION



VOLUME 66, 2019



# JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

## EDITORIAL STAFF

Rui-De Xue, Editor, Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL, 32092. [xueamcd@gmail.com](mailto:xueamcd@gmail.com)

Seth C. Britch, Assistant Editor, USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Dr. Gainesville, FL 32608. [Seth.Britch@ars.usda.gov](mailto:Seth.Britch@ars.usda.gov)

Derrick Mathias, Assistant Editor, University of Florida/IFAS, Florida Medical Entomology Laboratory, 200 9th St. SE, Vero Beach, FL 32962. [mathias@ufl.edu](mailto:mathias@ufl.edu)

Eva Buckner, Assistant Editor, University of Florida /IFAS, Florida Medical Entomology Laboratory, 200 9th St. SE, Vero Beach, FL 32962. [eva.buckner@ufl.edu](mailto:eva.buckner@ufl.edu)

New manuscripts of articles, operation or scientific notes, and annual meeting abstracts and page proofs should be sent to Editor by e-mail attachment at [xueamcd@gmail.com](mailto:xueamcd@gmail.com). The Editor will assemble the manuscripts to the assistant editor or subject editor to conduct the peer review process.

Copyright ©2019 by The Florida Mosquito Control Association, Inc.

Printed by E.O. Painter Printing Company, P.O. Box 877, De Leon Springs, FL 32130

## TABLE OF CONTENTS

### ARTICLES

<i>Aedes aegypti</i> oviposition differences among ornamental bromeliads with variable water levels Parker T. Brown, Molly E. Clark, Christopher S. Bibbs, and Rui-De Xue .....	1
<i>Aedes aegypti</i> survivorship on salt tolerant California landscape plants Christopher S. Bibbs, Jesse E. Crosier, Rui-De Xue, Gunter C. Muller, and Whitney A. Qualls .....	7
Evaluation of multiple trap types for the capture of vector mosquitoes of eastern equine encephalitis virus in Saint Johns County, Florida Daniel Dixon, Dena Autry, and Rui-De Xue .....	11
Control of adult & larval <i>Aedes albopictus</i> with attractive toxic sugar baits (Active ingredient: Cinnamonsesame oil) in North-eastern Florida Mohamed M. Traore, Amy Junnila, Edita E. Revay, Vasilij D. Kravchenko, Arlene Lahti, Jody M. Fiorenzano, Whitney A. Qualls, Daniel L. Kline, Yosef Schlein, John C. Beier, Rui-De Xue, and Gunter C. Muller .....	20
Field assessment of autodissemination of pyriproxyfen by container-inhabiting <i>Aedes</i> mosquitoes in Florida Barry W. Alto, Sara Ortiz, Keenan Wiggins, Cynthia C. Lord, and Nathan D. Burkett-Cadena .....	27
Laboratory toxicity of mosquito adulticides to the Asian tiger mosquito, <i>Aedes albopictus</i> and the honey bee, <i>Apis mellifera</i> Hussein Sanchez-Arroyo, Roberto M. Pereira, Daniel Dixon, Yong-Xing Jiang, Daniel Dixon, Rui-De Xue, and Philip G. Koehler .....	40
Evaluation of pyrethroid and botanical barrier insecticides against <i>Aedes albopictus</i> in the laboratory and field Steven T. Smoleroff, Christopher S. Bibbs, and Rui-De Xue.....	47
Semi-field ULV evaluation of an all-purpose botanical insecticide containing cedarwood and cinnamon oils against adult <i>Aedes aegypti</i> Christopher S. Bibbs, Kathy Shirley, Dena L. Autry, and Rui-De Xue.....	54
<b>OPERATIONS NOTE &amp; EQUIPMENT</b>	
Store time of permethrin in the truck-mounted ULV spray tanks caused degradation in the concentrations Rui-De Xue, Maia Tsikolia, Whitney A. Qualls and Uli Bernier.....	60
Evaluation of CDC light trap, BG sentinel trap, and MMX trap for the collection of salt marsh mosquitoes in Anastasia State Park, St. Augustine, Florida Daniel Dixon, Joseph D’Amato, and Rui-De Xue .....	64
Comparative efficacy of five permethrin/PBO 30-30 ground ULV insecticides against field collected adult <i>Aedes aegypti</i> , <i>Aedes taeniorhynchus</i> , and <i>Culex quinquefasciatus</i> in Manatee County, Florida Katie F. Williams, Eva A. Buckner, Ambyr L. Marsicano, Mark D. Latham, and Christopher R. Lesser .....	68

Continued

Evaluation of a new truck-mounted ULV spraying machine with *Bacillus thuringiensis* var. *israelensis* against larval *Culex quinquefasciatus*  
 Lei Lour, Marcia K. Gaines, and Rui-de Xue ..... 73

SUBMITTED ABSTRACTS OF THE 90TH ANNUAL MEETING

Smart phone application for submitting public service requests  
 James H. Richard Weaver and Rui-De Xue ..... 80

Analysis of the voltage-gated sodium channel (VGSC) target site mutation, L1014F, and pyrethroid resistance in *Culex quinquefasciatus* populations of Collier County, Florida  
 Keira J. Lucas, Kaci Mcoy, Caroline Weldon, and Rachel B. Bales ..... 81

Real-time PCR detection and operational response to West Nile virus positive mosquito pools at Sarasota County Mosquito Management  
 Chip Hancock ..... 81

Contrasting importance of state and in-house laboratory arbovirus testing of sentinel chicken and mosquito samples  
 Milton Sterling ..... 82

Frequent sugar feeding behavior by *Aedes aegypti* in Bamako, Mali makes them ideal candidates for control with attractive toxic sugar baits (ATSB): recent laboratory and field trials in Mali, West Africa  
 Presented by Gunter C. Muller ..... 82

Using mutant mosquitoes to find life-saving perfume  
 Joshua I. Raji, Nadia Melo, John Castillo, Sheyla Gonzalez, Valeria Saldana, Marcus Stensmy, and Mathew DeGennaro ..... 83

Laboratory evaluation of two new active ingredients for attractive toxic sugar baits (ATSB) against mosquitoes  
 Presented by Molly Clark ..... 83

Autocidal gravid traps (AGO) incorporation with attractants for control of gravid and host-seeking *Aedes aegypti*  
 Presented by Rui-De Xue ..... 84

Over 1,600 autocidal gravid trap (AGO) deployment in St. Augustine, 2018 and its impact on container inhabiting mosquitoes  
 Presented by Dena Autry ..... 84

Efficacy of plant saucers treated with residual larvicides against *Aedes albopictus* larvae under the semi and field conditions  
 Yongxing Jiang ..... 85

Flying under the influence: impaired fitness of susceptible, resistance, and field strains of *Aedes aegypti* after 60 second exposures to metofluthrin vapors  
 Christopher S. Bibbs ..... 85

Assessing the efficacy of operational mosquito control products through field trials  
 Casey Parker, Daviela Ramirez, and C. Roxanne Connelly ..... 86

Editors' acknowledgements

Instruction for contributors

## 2017-2018 THE FMCA PRESIDENTIAL ADDRESS

T. WAYNE GALE

Lee County Mosquito Control District, 15191 Homestead Rd.,  
Lehigh Acres, FL 33971



Greetings everyone and welcome to this year's meeting of the Florida Mosquito Control Association. It has been my pleasure and honor to serve as your President over this last year and FMCA is doing great things and is a wonderful organization to be a part of.

I would like to thank Andrea Leal for the great program she has put together for this meeting and all those who have submitted presentations. Also, a special thanks to all our exhibitors and sponsors for their participation and continued support of this organization and meeting. Please take time during this week to visit the vendor booths and displays, there are many great products and new information they have to share with you.

I want to take say a special thanks to Shelly Redovan, our Executive Director for coordinating this meeting and all she has done for our Association over the past several decades. Shelly is once again serving in a volunteer capacity to keep this organization running and has done so many times before. I can truly say that this organization would not be what it is today without Shelly, she has been the backbone and diligent caretaker of

FMCA for many years. We have had several challenging starts and stops with management of this Association over the years and each time Shelly has stepped in to pick up the pieces and keeps things going. There is a lot that goes into managing an organization like FMCA and the amount of work and effort is really equivalent to a full-time job. We are entering into a time when we must look forward and determine how this organization will be managed in the future so Shelly can enjoy her retirement. We are at a crossroad and must look carefully at our organization and budget to see if we can move ahead with hiring a professional management team, find talented and dedicated volunteers or some combination of both. But we must move toward transferring management responsibility from Shelly and find the best path forward. We will be working on this and do solicit your input and help in any way. Again, I want to thank Shelly for her hard work and dedication to FMCA.

It is hard to believe I have been serving with the Florida Mosquito Control Association for over 20 years. It is a tremendous organization and second to none when it comes to mosquito control. We have some challenges ahead and look forward to tackling those challenges and becoming even stronger and better for it.

I hope each of you has a great meeting this week, networking, learning and just having fun. Have a great meeting!

# 2016-2017 FMCA PRESIDENTIAL ADDRESS<sup>1</sup>: WHAT IS NEXT FOR POTENTIAL OUTBREAKS OF MOSQUITO-BORNE DISEASES?

RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

<sup>1</sup>Presentation given at the 89<sup>th</sup> Annual Meeting of the Florida Mosquito Control Association (FMCA) in Gaylord Palms, Kissimmee, FL, November 15, 2017

## INTRODUCTION



Welcome to the 89<sup>th</sup> annual meeting of the Florida Mosquito Control Association. Due to hurricane Mathew's damages, we relocated the meeting from Duck Key to Kissimmee this year. In the past 30 years, resurgent and/or emergent arboviruses, such as Rift Valley fever (RVF), dengue, yellow fever, Japanese encephalitis (JE), West Nile Virus (WNV), St. Louis encephalitis (SLE), chikungunya, and Zika have caused major global epidemics and public health problems. In Florida, several outbreaks of mosquito-borne diseases occurred in the past 20 years with the most recent outbreak of Zika in 2016-2017. These outbreaks have posed concerns for many people who are asking why this has happened now and which will be the next potential outbreak of mosquito-borne disease. I reviewed recent literature and attended several meetings to prepare this presentation and share the information with you.

## POSSIBLE REASONS

Global warming and climate changes alter the geographic and seasonal distributions of existing mosquitoes and mosquito-borne diseases, such as, malaria and dengue

fever. This is mainly due to expanding ranges of mosquitoes and an increase in blood feeding of infected mosquitoes. Expansion, as well as, invasive, and resurgent vector species of mosquitoes has been increasing. The rapid expansion of both vectors *Aedes aegypti* L. and *Ae. albopictus* Skuse may be caused by human mobility, increase in temperatures and continued urbanization, which drives most of the expansion of these species. Deforestation may also change the climate, as well as, mosquito and wildlife habitats. Approximately 18 million acres of forest have been lost per year in the past decade.

Global urbanization has increased in the past 20-30 years. The highest percentage of urbanization in the world is in South and North America and Australia. In addition, globalization is driven and constrained by economic process, technological developments, political influences, cultural and value systems, and social and natural environmental factors. This impacts the epidemiology of mosquito-borne diseases. Other factors include increases in international travel with the spread of mosquito-borne diseases, such as yellow fever, Zika, dengue fever, chikungunya, WNV, and malaria. The global transport networks by air, sea, and land, done through international travel, shipping routes, and other methods of transport, continue to expand in reach. The speed of travel and volume of passengers and goods carried also increases the global pathogens and vector mosquito traffic. Also, this increases the diseases spread and vector mosquitoes' expansion. This migration increases the risk

for reintroduction and localized outbreaks of mosquito-borne diseases.

Insecticide shortage and resistance are other challenges for prevention and control of mosquito-borne disease outbreaks. The cost of discovering new insecticides has gone up and the number of research based companies has gone down since the 1970's. Also, the high pyrethroid resistance we are seeing in many species of mosquitoes.

There is a lack of vaccines for most mosquito-borne diseases, such as malaria, filariasis, WNV, Eastern Equine Encephalitis (EEE), Chikungunya, Zika, and Dengue. The vaccines are available for yellow fever and Japanese type B Encephalitis for humans and vaccines for EEE and WNV are only available for animals. However, vaccinations are not given to all travelers or people in the epidemic countries. Yellow fever caused outbreaks in Angola and Japanese B Encephalitis in China in the past few years. Transmissions of other arboviruses and parasites occasionally happens due to blood transfusions, organ transplants, and unclean syringes. It is fortunate that the US Food & Drug Administration approved the Dengue vaccine, Dengvaxia, for humans in May 2019.

## MOSQUITO-BORNE DISEASES AND MAJOR VECTORS

Malaria, transmitted by *Anopheles* mosquitoes remains one of the worst health issues, with 219 million cases and 438,000 deaths in 2017, based on WHO's report. The most reported regions are African (92%) and Southeastern Asia. Filariasis, transmitted by *Culex*, *Anopheles*, *Aedes*, and *Mansonia* mosquitoes affected more than 200 million people, with more than 2 billion at risk in 112 countries before the year 2000. WHO launched the global program to eliminate lymphatic filariasis (LF), based on annual mass drug administration with albendazole and ivermectin combinations for everyone in the endemic countries. This has reduced LF prevalence to the elimination threshold of less than 1% in the majority of countries.

Dengue fever, transmitted by the container-inhabiting mosquitoes *Ae. aegypti* and

*Ae. albopictus*, is the world's most common mosquito-borne viral disease, annually affecting more than 400 million people worldwide and kills more than 20,000 people. Yellow fever, transmitted by *Ae. aegypti* is a viral disease, which is epidemic in tropical and subtropical areas of Africa and South America. Mosquitoes acquire the virus by feeding on infected humans or non-human primates and they can transmit the virus to other primates. People infected with yellow fever virus are infectious to mosquitoes. The transmission cycles are jungle (sylvatic), intermediate (savannah), and urban. Zika and Chikungunya, caused by Zika and chikungunya viruses, are transmitted by the bite of infected *Ae. aegypti* and *Ae. albopictus* mosquitoes.

St. Louis encephalitis (SLEV) and WNV are transmitted by *Culex* mosquitoes. Mosquitoes become infected when they feed on infected birds. Infected mosquitoes then spread SLEV or WNV to people and other animals by biting them. People and horses are incidental hosts. Other arboviruses, for example, EEE is transmitted by *Culiseta melanura*, RVF is transmitted by *Aedes* and *Culex* mosquitoes.

Currently, we know the following possible arboviruses that are transmitted by vector mosquitoes: Bunyaviridae with 5 genera and 248 viruses, Flaviviridae with 1 genera and 53 viruses, Roviridae with 2 genera and 77 viruses, Rhabdoviridae with 12 genera and 68 viruses, Togaviridae with 1 genera and 28 viruses, Orthomyxoviridae with 1 genera and 3 viruses, Arenaviridae with 1 genera and 1 virus, and Poxviridae & unclassified with 2 genera and 14 viruses. There are still many unknown viruses.

## TRANSMISSION TYPES AND POTENTIAL OUTBREAKS OF MAJOR DISEASES

There are several types of transmission cycles for mosquito-borne diseases. The major transmission cycle is Birds-Mosquito-Birds. Humans and animals are incidental hosts for mosquito-borne diseases, such as WNV, SLE, and EEE. The other transmission cycle type is, human-mosquito-human,



for mosquito-borne diseases such as yellow fever, dengue fever, chikungunya, and Zika.

The diseases transmitted by the human-mosquito-human transmission cycle, have the most potential for outbreaks, regardless if they are old or new diseases. In the past 20 years, Florida experienced outbreaks of malaria (2003-2004), WNV (2002-2003 & 2012), dengue fever (2009-2010), Chikungunya (2014-2015) and Zika (2016-2017). The resurgence and spread of *Ae. aegypti*, the most important vector for Dengue, Chikungunya and Zika is a major concern. Other potential vectors, including *Ae. albopictus* and others of Mayaro virus may also cause problems. The diseases transmitted by the Birds-Mosquito-Birds transmission cycle and other multiple transmission types have the second highest potential for outbreaks, such as WNV, RVF, and JE.

#### WHAT THE FMCA HAS DONE IN 2016-2017

The FMCA Board approved the Florida Department of Agriculture and Consumer Service (FDACS)'s Zika vector control documentation in November 2016. FMCA held its first time ever news release about Zika prevention and control at the Tallahassee Legislation Day in March 2017. The FMCA Board supported new technology for the control of Zika mosquitoes to be used in Florida.

FMCA trained numerous mosquito control professionals through the Fly In Class in the middle of January, the Dodd short course

in late January, FDACS's 7 regional meetings and workshops, the Entomologist and Biologist workshop, annual meeting, FDACS & American Mosquito Control Association (AMCA) regional workshops, and AMCA's training hub host's certification training for trainers at the Anastasia Mosquito Control District (AMCD) in July, August, and October, 2017. FMCA collaborated with and supported FDACS and the Florida Department of Health (FDOH)'s weekly teleconference, regional meetings, and Governor's regional round table meetings.

FMCA collaborated with the CDC Southeastern Center for Excellence in Vector-borne Diseases and AMCA for training Florida mosquito control professionals. In 2017, there was only 1 locally acquired transmission of Zika due to the many efforts to control mosquito-borne diseases.

#### ACKNOWLEDGEMENTS

It is a great pleasure to serve the FMCA as your Association President. Thank you very much for your support and your votes in November 2014. Thank you to the Executive Director, Shelly Redovan, all Board members, committee chairs, committee members, and the Anastasia Mosquito Control District's Board of Commissioners' for allowing me to provide the service and thank you to all of AMCD's employees for their support and C. Hall, E. Zeszutko, and C. Efstathion for editing and proof reading this manuscript.

# **AEDES AEGYPTI OVIPOSITION DIFFERENCES AMONG ORNAMENTAL BROMELIADS WITH VARIABLE WATER LEVELS**

PARKER T. BROWN, MOLLY E. CLARK, CHRISTOPHER S. BIBBS,  
AND RUI-DE XUE

<sup>1</sup>Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Subject Editor: Derrick Mathias

## ABSTRACT

*Aedes aegypti* (L.) have recurrently been emphasized as a critical vector amidst the emergence, and re-emergence, of various anthroponoses. Bromeliads have been incriminated as an *Ae. aegypti* refuge. To investigate this, common ornamental bromeliads in the genera *Guzmania*, *Neoregelia*, and *Vriesea* were used in oviposition bioassays with *Ae. aegypti*. No choice assays were conducted with all three plant types alongside variations in water level, approximated as low, medium, or high based on a 25%, 50%, and 75% capacity. Gravid *Ae. aegypti* tended to deposit eggs in leaf axils of *Neoregelia* more than the central bowl, or tank. In contrast, *Guzmania* collected more eggs in the central bowl, and collected the most eggs when water levels were high. No other trends based on water level were apparent across the other types. *Vriesea* collected few eggs regardless of location or water level. *Neoregelia* collected more eggs overall, implicating this type of bromeliad is potentially suitable to *Ae. aegypti*. This was a preliminary investigation into the role of bromeliads for *Ae. aegypti* oviposition. However, even when mosquitoes were not allowed a choice, there were clear differences in egg deposition between bromeliad types. More information is needed that partitions the role of different bromeliads for the vectors of concern. Future operations and education should prioritize the bromeliads that have a clear connection with the target mosquitoes, particularly in light of controversy about the role bromeliads may play in mosquito-borne disease outbreaks.

Key Words: bromeliads, oviposition, mosquito, behavior

## INTRODUCTION

Container-inhabiting mosquitoes, such as *Aedes aegypti* (L.), are peridomestic to human populations, proliferating in artificial containers such as trash, bird baths, open pipes, and buckets (Ngugi et al. 2017; Nordin et al. 2017). Source reduction programs are the main strategy for eliminating common artificial oviposition sites (Kittiyapong et al. 2008; Nordin et al. 2017). Unfortunately, the persistence of container-inhabiting mosquitoes is due in part because they also use natural containers (Nordin et al. 2017). Although container-inhabiting mosquitoes are highly productive in tires or plastic receptacles (Faraji and Unlu 2016; Unlu et al. 2016), the decline of Florida bromeliad specialists, such as *Wyeomyia vanduzeei* and *W. mitchellii* (O'Meara et al. 1995; Lounibos et al. 2003), and subsequent shifts in distribution in north Florida have allowed the en-

croachment of the aforementioned invasive *Aedes* species into bromeliads (O'Meara et al. 1995; Xue et al. 2018). Although artificial containers are still the primary concern for these *Aedes* species, the re-emergence of *Ae. aegypti* in the same areas where *Wyeomyia* have declined have increased the need to understand how container-mosquito oviposition ecology intersects with bromeliads.

Ornamental bromeliads have a wide variation in size and color, which leads them to being a common plant in both rural and urban environments throughout Florida, particularly southern Florida (Wilke et al. 2018). Their overlapping bowl-like axils collect water, providing an essential role in vegetative environments as a drinkable water source and also shelter for many insect and amphibious species, including some mosquitoes. Additionally, in urban settings ornamental bromeliads can be a coveted landscaping or decorative plant in both resi-

dential and public places, which can lead to difficulty in cultural control when home and business owners are not complicit in source reduction programs (Unlu et al. 2013; Wilke et al. 2018). Pesticide pressure among easy to diagnose harborages (Wilke et al. 2018) and skip-oviposition behavior (Colton et al. 2003) leads container-inhabiting mosquitoes to occupy otherwise atypical oviposition environments (Ramasamy et al. 2011; Chitolina et al. 2016).

The role that bromeliads play as a refuge when selective pressure (e.g., pesticide application) is high should be investigated to clarify whether *Ae. aegypti* is linked with particular bromeliad types. Key sites for container-inhabiting mosquitoes tend to require nutrient rich water and partial or indirect sunlight. Thus, bromeliads in the genus *Neoregelia* can become niches for *Ae. aegypti* because of large flowers that decompose in their water impoundments, possibly over-enriching the water to a degree that may exclude specialist competitors (J. H. Frank, pers. comm). In sampling throughout the jurisdiction of the Anastasia Mosquito Control District of St. Johns County, FL (AMCD), *Neoregelia* and *Guzmania* are commonly encountered bromeliad genera (Xue et al. 2018). *Vriesea* is less common, but is persistently available through local landscape providers. To take the first steps in investigating domestic mosquito preferences in bromeliads, we examined the oviposition of *Ae. aegypti* in *Neoregelia*, *Guzmania*, and *Vriesea* with three water levels.

## MATERIALS AND METHODS

*Aedes aegypti*, 1952 Orlando strain, were acquired from the United States Department of Agriculture, Agricultural Research Service, Center for Medical and Veterinary Entomology. Mosquitoes were reared in the AMCD insectaries at 26.7°C, 80% RH, and 14:10 L:D photoperiod. Larvae were fed a 25 mg of a mixture of 1:1 yeast:liver powder bi-daily. Adult mosquitoes were kept on a diet of 10% sucrose solution. Once over seven days old, mosquitoes were blood fed and set

aside for 72 h to become gravid before use in bioassays.

Ten gravid mosquitoes were aspirated into a tented cage (BugDorm 1462W, Bioquip Products Inc., Rancho Dominguez, CA) that contained 10% sucrose solution and one of either a *Neoregelia*, *Guzmania*, or *Vriesea* bromeliad (Fig. 1). The total water-holding capacity across the bowl and axils for each plant type averaged 194 ml, 68 ml, and 64 ml for *Neoregelia*, *Guzmania*, and *Vriesea*, respectively. The bromeliads were coded as low (for ~25%), medium (for ~50%), or high (for ~75%) based on the qualitative water level maintained in the center and two prominent leaf axils. All water levels in each bromeliad were tested concurrently in no-choice assays where they did not have access to the other bromeliads or alternative breeding sites.

Once mosquitoes were added to the tents, they remained there for 3 days to allow sufficient opportunity to oviposit. Water levels were maintained at their respective assignments by manually adding reverse osmosis water to the bowl or axils both for the initial fill and daily during the bioassay. Upon concluding the bioassay, the central bowl and two prominent leaf axils were inspected for mosquito eggs. Larvae were then reared out from the eggs inside the bromeliad and cared for until adulthood using the same rearing conditions as the insectary. The assay was repeated three times, each time with new water. The difference in egg deposition between the bowl or leaf axils and low-, medium-, or high-water level was not distributed normally. Therefore, data were analyzed by bromeliad type using Kruskal-Wallis non-parametric ANOVA (Kruskal and Wallis 1952) and Steel-Dwass all pairs post-hoc test (Critchlow and Fligner 1991).

## RESULTS AND DISCUSSION

There were no overarching trends in the number of eggs collected in the central bowl of the bromeliads when analyzed by the differences in water level (Fig. 2). For both *Neoregelia* and *Vriesea*, low- and high-water levels resulted in comparable amounts of eggs deposited, while medium-water lev-

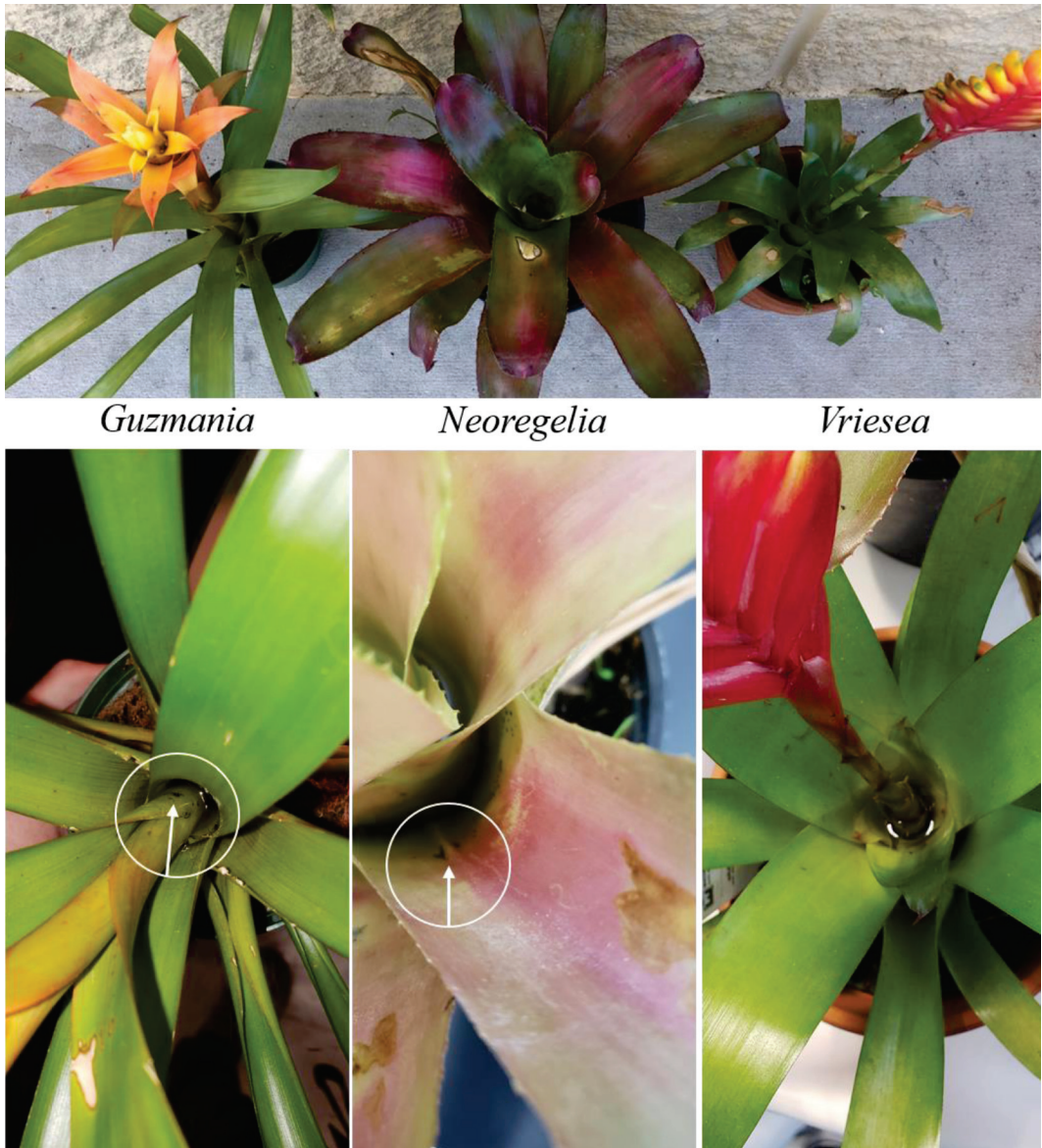


Figure 1. Common ornamental bromeliad types used in oviposition bioassays. Top: From left to right, *Guzmania*, *Neoregelia*, and *Vriesea*. Bottom: Post-bioassay images showing egg deposition within the center part (*Guzmania*), leaf axil (*Neoregelia*), or not at all (*Vriesea*).

els resulted in a visual but non-significant trend of fewer eggs than the other water levels. With *Guzmania*, low- and medium-water levels did not result in significantly different amounts of eggs deposited. The high-water level in *Guzmania* collected a comparable mean of eggs to *Neoregelia* (Fig. 2) with no statistical separation between the two plant types. When examining egg deposition by the central bowl of the plant or

the two prominent leaf axils, regardless of water level, there was a visual, but not statistical, trend for *Guzmania* to have more eggs deposited in the central bowl than in the leaf axils (Fig. 2). Similarly, *Neoregelia* tended to have more eggs deposited in leaf axils than the central bowl, but power was insufficient to statistically support the observation. Egg deposition in *Vriesea* did not favor either particular location.

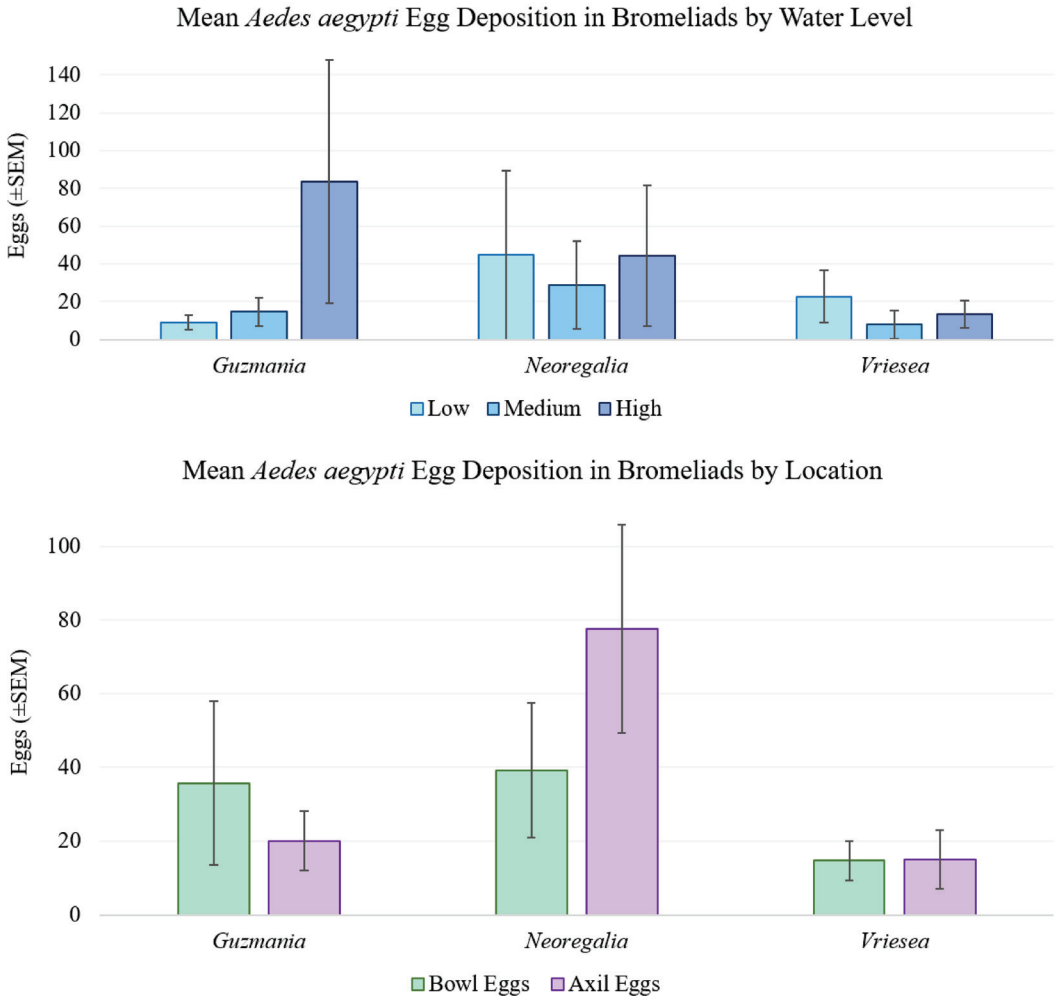


Figure 2. Trends in mean percent egg deposition by *Aedes aegypti* (L.) based on discriminating factors analyzed from non-choice tests, represented with standard error of the mean. Top: Deposition based on low- (~25%), medium- (~50%), or high-water (~75%) lines in the respective plant. Bottom: Deposition based on the central bowl or leaf axil in the respective plant.

Analyzing the total eggs per plant, regardless of water level or location, resulted in no statistical differences between *Guzmania*, *Neoregalia* or *Vriesea* with eggs totaling 321, 353, and 132, respectively. Though not significant between plants, it was observed that an average of 21%, 25%, and 41% of eggs in *Guzmania*, *Neoregalia*, and *Vriesea*, respectively, were floating atop the surface of the water rather than affixed directly to a leaf. Beyond that, there was an average of 68%, 20%, and 25% of assayed *Ae. aegypti* being found deceased in the impounded water within *Guzmania*, *Neoregalia*, and *Vriesea*, respectively. Post bioassay, larvae were

successfully reared to adulthood in all plants within 14 days without requiring any food input. There were no apparent differences among plants for the success of larval rearing, given the artificial conditions of the bioassay.

Although investigated through a series of no-choice assays, it was interesting to see that *Ae. aegypti* deposited eggs in all three types despite that bromeliads are considered inhospitable rearing environments for non-specialists (Lounibos et al. 2003; O'Meara et al. 2003; Mocellin et al. 2009; Lopez et al. 2011). Among the bromeliad types tested, it superficially appeared that

egg deposition was higher in *Neoregelia*. Other reports have tended not to specifically justify whether a certain genus of bromeliad is more prone to harboring invasive vector mosquitoes. If *Neoregalia*, or any one genus, were more prone to oviposition by *Ae. aegypti*, then source reduction could be more discriminatory about bromeliads that are considered harmful for public health. It is noteworthy that *Guzmania* harbored more eggs in the central portion than the leaf axils, but we attribute this to a difference in structure. The *Guzmania* used in these bioassays contained a central stalk composed of several interwoven leaf blades. Particularly with the finding that *Guzmania* contained more eggs at the high-water level, we believe that simply increased the surface area along the central stalk to which *Ae. aegypti* could adhere eggs (Fig. 1). *Vriesea* did not appear to be as suitable for oviposition, as even despite no preference, the fewest total eggs were deposited in either the central bowl or leaf axils. However, a caveat remains that the experiments were performed with colony mosquitoes. It is therefore possible that altering our experimental design to use mosquitoes reared from wild-collected eggs and increasing replication would have yielded different results. In addition, complementing experiments with sampling eggs from the same bromeliad species in the field may provide additional insight. Moreover, even though fewer eggs were found in *Vriesea* plants, they were still positive for the presence of *Ae. aegypti* eggs.

It is puzzling that such a high proportion of eggs were found on the surface of the water, as opposed to in available crevices. Historical study shows lower humidity to correlate with higher water oviposition (Chadee et al. 1995). However, we believe additional factors were more influential, such as texture. Smooth oviposition surfaces have been shown to encourage laying eggs on the water, or may lead to avoiding the oviposition site altogether (Madeira et al. 2002). *Aedes aegypti* have been reinforced as preferring rough, rugose (wrinkled) lining in oviposition sites

over smooth surfaces (Swan et al. 2018). We believe the leaves of certain bromeliads may lack sufficient texture for *Ae. aegypti* oviposition, which could also explain the post-bioassay mortality of adults that appeared to have fallen into the water. The lower mortality and water oviposition in *Neoregelia* assays may point to there being less of an obstacle through texture. But this may be better examined in future study through a combination of choice assays with artificial and natural containers, as well as possible substitution assays where a bromeliad leaf is used in lieu of standard substrates inside of an oviposition cup.

Bromeliad structure and prevalence in peridomestic landscapes is believed to contribute to vector risks (Wilke et al. 2018). However, historical study contradicts the importance that is attributed to bromeliads for *Ae. aegypti* (Frank and Curtis 1977; Frank et al. 1988; Mocellin et al. 2009). Therefore, we believe that such mixed findings are the result of bromeliad-specific differences that confound our understanding of bromeliads in the oviposition ecology of peridomestic vectors. Bromeliad utilization also may be a geographically linked phenomenon, as the plants may not be preferred but in some cases they may be the most abundant option in the landscape. Our current investigations are a preliminary attempt at understanding oviposition differences in natural containers, but show that, even in absence of top-down pressures, *Ae. aegypti* may not interact with all bromeliads equally. To resolve misconceptions, we propose that available source reduction education should begin prioritizing specific types of bromeliads that are liable to cause risks, which appear to be particular ornamental varieties (Wilke et al. 2018).

#### ACKNOWLEDGMENTS

We would like to thank Kathy Shirley for assistance in caring for bromeliads and collecting eggs for this project. All work was conducted at the Anastasia Mosquito Control District, St. Augustine, FL with no external support.

## REFERENCES CITED

- Chadee DD, Corbet PS, Tailbot H. 1995. Proportions of eggs laid by *Aedes aegypti* on different substrates within an ovitrap in Trinidad, West Indies. *Med Vet Entomol* 9:66–70.
- Chitolina RF, Anjos FA, Lima TS, Castro EA, Costa-Ribeiro MCV. 2016. Raw sewage as breeding site to *Aedes (Stegomyia) aegypti* (Diptera, Culicidae). *Acta Trop* 164:290–296.
- Colton YM, Chadee DD, Severson DW. 2003. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Med Vet Entomol* 17:195–204.
- Critchlow DE and Fligner MA. 1991. On distribution-free multiple comparisons in the oneway analysis of variance. *Commun Stat Theory Methods* 20:127–139.
- Faraji A, Unlu I. 2016. The eye of the tiger, the thrill of the fight: effective larval and adult control measures against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. *J Med Entomol* 53:1029–1047.
- Frank JH, Curtis GA. 1977. On the bionomics of bromeliad-inhabiting mosquitoes. III. The probable strategy of larval feeding in *Wyeomyia vanduzeei* and *W. medioalbipes*. *Mosquito News* 37:33–43.
- Frank JH, Stewart JP, Watson DA. 1988. Mosquito larvae in axils of the imported bromeliad *Billbergia pyramidalis* in southern Florida. *Florida Entomol* 71:33–43.
- Kittayapong P, Yoksan S, Chansang U, Chansang C, Bhumiratana A. 2008. Suppression of dengue transmission by application of integrated vector control strategies at sero-positive GIS-based foci. *Am J Trop Med Hyg* 78:70–76.
- Kruskal WH, Wallis A. 1952. Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 47:583–624.
- Lopez LCS, Silva EGB, Beltrão MG, Leandro S, Barbosa JEL, Beserra EB. 2011. Effect of tank bromeliad micro-environment on *Aedes aegypti* larval mortality. *Hydrobiologia* 665:251–261. DOI: 10.1007/s10750-011-0605-8
- Lounibos LP, O'Meara G, Nishimura N. 2003. Interactions with native mosquito larvae regulate the production of *Aedes albopictus* from bromeliads in Florida. *Ecol Entomol* 28:551–558.
- Madeira NG, Macharelli CA, Carvalho LR. 2002. Variation of the oviposition preferences of *Aedes aegypti* in function of substratum and humidity. *Mem Inst Oswaldo Cruz* 97:415–420.
- Mocellin MG, Simões TC, Nascimento TF, Teixeira ML, Lounibos LP, Oliveira RL. 2009. Bromeliad-inhabiting mosquitoes in an urban botanical garden of dengue endemic Rio de Janeiro—are bromeliads productive habitats for the invasive vectors *Aedes aegypti* and *Aedes albopictus*? *Mem Inst Oswaldo Cruz* 104:1171–1176.
- Ngugi HN, Mutuku FM, Ndenga BA, Musunzaji PS, Mbakaya JO, Aswani P, Irungu LW, Mukoko D, Vulule J, Kitron U, LaBeaud AD. 2017. Characterization and productivity profiles of *Aedes aegypti* (L.) breeding habitats across rural and urban landscapes in western and coastal Kenya. *Parasit Vectors* 10:331.
- Nordin O, Ney TG, Ahmad NW, Benjamin S, Lim LH. 2017. Identification of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) breeding habitats in dengue endemic sites in Kuala Lumpur Federal Territory and Selangor State, Malaysia. *SE Asian J Trop Med* 48:786–798.
- O'Meara, GF, Evans, LF, Gettman, AD, Patteson, AW. 1995. Exotic tank bromeliads harboring immature *Aedes albopictus* and *Aedes bahamensis* (Diptera: Culicidae) in Florida. *J Vector Ecol* 20:216–224.
- Ramasamy R, Surendran SN, Jude PJ, Dharshini S, Vinobaba M. 2011. Larval development of *Aedes aegypti* and *Aedes albopictus* in peri-urban brackish water and its implications for transmission of arboviral diseases. *PLoS Negl Trop Dis* 5:e1369.
- Swan T, Lounibos LP, Nishimura N. 2018. Comparative oviposition site selection in containers by *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) from Florida. *J Med Entomol* 55:795–800
- Unlu I, Farajollahi A, Strickman D, Fonseca DM. 2013. Crouching tiger, hidden trouble: urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. *PLoS ONE* 8:e77999.
- Unlu I, Klingler K, Indelicato N, Faraji A, Strickman D. 2016. Suppression of *Aedes albopictus*, the Asian tiger mosquito, using a 'hot spot' approach. *Pest Manag Sci* 72:1427–1432.
- Wilke AB, Vasquez C, Mauriello PJ, Beier JC. 2018. Ornamental bromeliads of Miami-Dade County, Florida are important breeding sites for *Aedes aegypti* (Diptera: Culicidae). *Parasit Vectors* 11:283.
- Xue RD, Lippi C, Drake L. 2018. Species composition of mosquitoes and invertebrates in common bromeliad plant axils (Family: Bromeliaceae) and the plant impacts on survival of vector mosquito, *Aedes albopictus* (Diptera: Culicidae). *China Trop Med* 18:6–21.

# **AEDES AEGYPTI SURVIVORSHIP ON SALT TOLERANT CALIFORNIA LANDSCAPE PLANTS**

CHRISTOPHER S. BIBBS<sup>1</sup>, JESSE E. CROSIER<sup>1,2</sup>, RUI-DE XUE<sup>1</sup>,  
GUNTER C. MÜLLER<sup>3,4</sup>, AND WHITNEY A. QUALLS<sup>5,6</sup>

<sup>1</sup>Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

<sup>2</sup>Current address: St. Johns County Department of Health,  
200 San Sebastian View, St. Augustine, FL 32084

<sup>3</sup>Malaria Research and Training Center, Faculty of Medicine, Pharmacy and  
Odonto-Stomatology, University of Bamako, BP 1805, Bamako, Mali

<sup>4</sup>Department of Microbiology and Molecular Genetics,  
IMRIC, Kuvin Centre for the Study of Infectious and Tropical Diseases,  
Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel

<sup>5</sup>University of Miami, Miller School of Medicine, 1600 Northwest 10<sup>th</sup> Avenue #1140,  
Miami, FL 33136

<sup>6</sup>Current address: Texas Department of State Health Services,  
Arbovirus and Entomology, 1100 West 49<sup>th</sup> Street, Austin, TX 78714

Guest Editor: M. Miah

## ABSTRACT

*Aedes aegypti* has expanded its range in the United States to include various arid and desert geographies, with notable introduction into various parts of California. Because resources are limited in arid environments, it is currently an important topic to understand how *Ae. aegypti* interacts with its surrounding environment for survival and proliferation. Three common plant species in peridomestic landscape, i.e., salt cedar (*Tamarix aphylla*), arrow weed (*Pluchea sericea*) and four wing saltbush (*Atriplex canescens*), were collected for survival bioassays to understand how *Ae. aegypti* is persisting in arid, chaparral landscapes in California, USA. These three plant-species along with a 10% sucrose solution (positive control) and reverse osmosis water solution (negative control-) were added to cages of *Ae. aegypti* to assess their survival at 24h, 48h, and 96h. It was found, in comparison with the negative control and four wing salt bush, that arrow weed and to a lesser extent salt cedar, promoted survival of *Ae. aegypti* in the first 24h. After the first day, only arrow weed significantly supported mosquito survival out to 96h as compared to the controls. Arrow weed and salt cedar are both riparian plants producing some nectaries which could be energy resources provided through stem sap or nectar to *Ae. aegypti* amidst peridomestic chaparral in California.

Key Words: *Aedes aegypti*, survivorship, *Pluchea sericea*, *Tamarix aphylla*, *Atriplex canescens*

## INTRODUCTION

*Aedes aegypti* (L.) is a potential vector for several globally significant emergent and re-emergent viral pathogens (Paules and Fauci 2017). In recent years, intermittent introductions of *Ae. aegypti* L. into the arid southwest (Madon et al. 2002) eventually resulted in breeding populations in the

desert chaparral of California, USA (Gloria-Soria et al. 2014; Henke 2016). Arid lands are not inherently considered ideal habitats for *Ae. aegypti*, but their ecology, as with other mosquitoes, is intrinsically dependent on the availability of plants as an oasis of resources. Particularly, the need to feed on sugar is limiting for both male and female mosquitoes (Yuval 1992). Hence, the



survivorship of *Ae. aegypti* regarding the viability of chaparral plants in peridomestic environments is the primary interest of this study.

Preliminary land cover analysis was performed by surveying peridomestic chaparral in Coachella Valley for common plants associated with urban landscapes. It was found that three plant species like salt cedar (*Tamarix aphylla*), arrow weed (*Pluchea sericea*) and four wing saltbush (*Atriplex canescens*) were most common in that area.

Studies in arid and ecologically transitional environments have shown mosquitoes can acquire sugar from flowers, extra-floral nectars, honeydew, fruits, and by directly piercing soft plant tissues to extract sap (Yuvall 1992; Müller et al. 2010; 2011). Additionally, the arid tree *Tamarix jordanis* is favored by *Culex pipens* L. for sugar feeding (Schlein et al. 2008). It stands that salt cedar, arrow weed, and four wing saltbush may provide amenable sugar resources for the survival of *Ae. aegypti* in an otherwise resource deprived environment. In the present study, therefore, survival bioassays were conducted with some plant species to investigate the impact of these plants on survivorship of invasive *Ae. aegypti*, and also to determine the possibility of *Ae. aegypti* forage on these plant species for survival.

## MATERIALS AND METHODS

*Aedes aegypti* mosquitoes were collected from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology and reared in insectaries maintained at  $26.6 \pm 1^\circ\text{C}$ ,  $80 \pm 0.5\%$  relative humidity, and a 14L:10D photoperiod. Survival bioassays were conducted with 20 adult females of *Ae. aegypti* (5-7d and non-blood fed) that were sugar-starved for 24h prior to the experiments. The tested plant species i.e. salt cedar, arrow weed, and four wing saltbush were collected from native peridomestic landscapes in Indio, CA, USA. The cuttings from these plants were stored in 900 ml of reverse osmosis (RO) water in a glass bottle for testing.

The general bioassay design required modifying a 5-gallon bucket by cutting open a 30 cm x 30 cm opening in the face and securing a 60 cm length of cylindrical stockinet to the opening as an entry tunnel. The top of the bucket was enclosed with 2 mm hardware cloth mesh (Fig. 1). A set of three plant cuttings of the same plant for a given species were admitted to the bucket and a batch of 20-mosquitos was aspirated into the cage. After securing the cage by tying off the stockinet and housing the cages were placed in insectary rearing conditions, bioassays were conducted for 96h. Each plant was evaluated three times in four replicates per evaluation period. For each replicate, there was a positive control and a negative control. The positive control consisted of cotton balls soaked with 10% sucrose solution and the negative control consisted of cotton balls soaked in RO water. Once bioassays started, *Ae. aegypti* survival was documented at 24h, 48h, and 96h for each treatment and control. Survivorship at each time of observation was averaged across replicates but



Figure 1. Bucket cage design. Top: A 5-gallon bucket with 30 x 30 cm front panel removed. The front face was enclosed by securing a 60 cm length of cylindrical stockinet. Bottom: Salt cedar cuttings nested in water for conducting a bioassay.

within respective treatments and analyzed using repeated measures ANOVA and post hoc paired t-tests.

## RESULTS AND DISCUSSION

The mean percentage *Ae. aegypti* survival at 24h, 48h, and 96h during exposure to treatments and control are summarized and presented in Figure 2. *Ae. aegypti* survived to a significantly greater degree on arrow weed and salt cedar at 24h, compared to the negative control ( $F = 14.3$ ,  $df = 4$ ,  $p < 0.0014$ ). At 48h, significantly more survival was observed in arrow weed than negative controls ( $F = 6.60$ ,  $df = 4$ ,  $p < 0.0148$ ), with salt cedar no longer having significantly different survival than the negative control. At 96h, arrow weed remained the only treatment group to have significant survival, as compared to negative controls ( $F = 2.82$ ,  $df = 4$ ,  $p < 0.0307$ ). A significant proportion of *Ae. aegypti* were not observed to survive on four wing saltbush at any observation time.

Arrow weed, and to a lesser extent salt cedar, allowed *Ae. aegypti* to survive significantly more in the first 24h of the bioassay than being

without sugar resources. However, arrow weed was the only significant treatment after the first 24h. The plant was not openly flowering and no direct observations could be identify the feeding/probing site during the bioassay. Arrow weed appeared to be a moderately fleshy, soft tissue plant on both stems and leaves. It is possible that *Ae. aegypti* enhanced survival is an example of their ability to draw nutrients directly from the plant vegetative structures. Salt cedar had small florets adorning the terminal ends of the cuttings, which may explain the increased survivorship on salt cedar for the first 24h. Given the arid landscape nature of the plants, it is possible the holding conditions were detrimental to the salt cedar, which may have reduced the viability of the flowers past the first 24 hours. This plant was also the only one in a tree growth form. In contrast, four wing saltbush, was similarly fleshy and green as in the case of arrow weed. The plant was somewhat woodier than arrow weed, so it is possible that there is something similar between salt cedar and four wing saltbush that prevented them from being viable to the survivorship of *Ae. aegypti* for the duration of the bioassay.

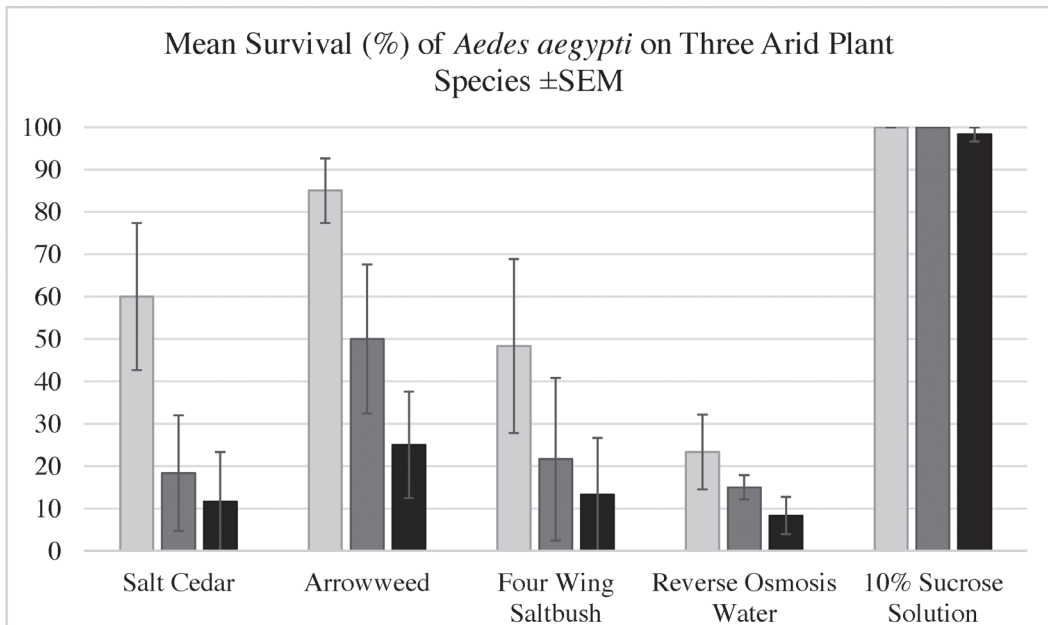


Figure 2. Cluster bar graph showing the mean percent survival of adult female *Aedes aegypti* (L.) when exposed to salt cedar (*Tamarix aphylla*), arrow weed (*Pluchea sericea*) and saltbush (*Atriplex canescens*); a reverse osmosis water (negative control); and a 10% sucrose solution (positive control). Values are displayed with standard error of the mean as I-bars.

With that context, the utility of arrow weed as a harborage or sugar resource may be contingent on the associated sub-populations of *Ae. aegypti* having heat, salt, or xeric tolerance. Population genetics has now incriminated *Ae. aegypti* in multiple introductions into California (Pless et al. 2017). These introductions are thought to have stemmed from south-central and south-western desert populations of *Ae. aegypti* in North America (Pless et al. 2017), and reflect that the established breeding populations of this mosquito species in southern California may already have been selected for fitness in arid lands. Additionally, all three plant species have differentiating qualities that may prove harsh for mosquitoes. Four wing saltbush is alkaline, having various medicinal uses for native desert tribes (Camazine and Bye 1980) that may in turn indicate it is unappealing or toxic to *Ae. aegypti*. In contrast, salt cedar is highly resistant to salt and alkaline conditions and develops in riparian areas (Griffin et al. 1989), possibly indicating this as a microhabitat suitable for *Ae. aegypti*. Arrow weed is also a salt tolerant riparian plant in coastal scrublands in the southern coastal basin of California (Boufford 1997), and may be the best indicator of potential for *Ae. aegypti* to harbor and survive. Regardless of habitat indication, *Ae. aegypti* can survive for several days with only arrow weed as a sugar resource, implying a relationship in arid lands that may account for how *Ae. aegypti* survives in peridomestic chaparral in California, and this information may benefit for managing *Ae. aegypti* in arid lands.

#### ACKNOWLEDGEMENTS

This research was funded by the Coachella Valley Mosquito and Vector Control Dis-

trict. Technical guidance and support was provided by Gregory White and Jennifer Gibson-Corrado.

#### REFERENCES CITED

- Boufford DE. 1997. Asteraceae – *Pluchea*. In: Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico*. Vol. 19, pp. 3-70. Oxford University Press, Oxford, UK.
- Camazine S, Bye RA. 1980. A study of the medical ethnobotany of the Zuni Indians of New Mexico. *J Ethnopharmacology* 2: 365-388.
- Gloria-Soria A, Brown JE, Kramer V, Yoshimizu MH, Powell JR. 2014. Origin of the dengue fever mosquito, *Aedes aegypti*, in California. *PLoS Negl Trop Dis* 8: e3029.
- Griffin GF, Smith DM, Morton SR, Allan GE, Masters KA, Preece N. 1989. Status and implications of the invasion of tamarisk (*Tamarix aphylla*) on the Finke River, Northern Territory, Australia. *J Environ Manag* 29: 297-315.
- Henke, JA. 2016. Fighting *Aedes aegypti* in the California desert. *Wing Beats* 27:23-27.
- Madon MB, Mulla MS, Shaw MW, Klueh S, Hazelrigg JE. 2002. *Aedes albopictus* (Skuse) introduction into southern California and potential for its establishment. *J Vect Ecol* 27: 149–154
- Müller GC, Xue RD, Schlein Y. 2010. Seed pods of the carob tree *Ceratonia siliqua* are a favored sugar source for the mosquito *Aedes albopictus* in coastal Israel. *Acta Trop* 116: 235–239
- Müller GC, Xue RD, Schlein Y. 2011. Differential attraction of *Aedes albopictus* in the field to flowers, fruits, and honeydew. *Acta Trop* 118:45–49
- Paules CI, Fauci AS. 2017. Yellow fever — Once again on the radar screen in the Americas. *N Engl J Med* 376: 1397-1399.
- Pless E, Gloria-Soria A, Evans BR, Krame V, Bolling BG, Tabachnick WJ, Powell JR. 2017. Multiple introductions of the dengue vector, *Aedes aegypti*, into California. *PLoS Negl Trop Dis* 11: e0005718.
- Seeger KE, Scott JM, Muller GC, Qualls WA, Xue RD. 2017. Effect of common species of Florida landscaping plants on the efficacy of attractive toxic sugar baits against *Aedes albopictus*. *J Am Mosq Control Assoc*. 33:139-141.
- Schlein Y, Müller GC. 2008. An approach to mosquito control: using the dominant attraction of flowering *Tamarix jordanis* trees against *Culex pipiens*. *J Med Entomol* 45: 384–390
- Yuval B. 1992. The other habit: sugar-feeding by mosquitoes. *Bull Soc Vector Ecol*, 17: 150-156.

# EVALUATION OF MULTIPLE TRAP TYPES FOR THE CAPTURE OF VECTOR MOSQUITOES OF EASTERN EQUINE ENCEPHALITIS VIRUS IN SAINT JOHNS COUNTY, FLORIDA

DANIEL DIXON<sup>1</sup>, DENA AUTRY, AND RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Current address: USDA/CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608

Subject Editor: Derrick Mathias

## ABSTRACT

Eastern equine encephalitis virus (EEEV) is a highly dangerous alphavirus vectored by multiple mosquito species in the United States. Vector surveillance and control is used to prevent the spread of EEEV, so highly efficient and attractive traps are needed to accurately assess mosquito abundance. Mosquitoes can be captured in various physiological states (host-seeking, gravid, resting, etc.), depending on what trap type is used. This study analyzed 6 trap types to determine which captured the most EEEV vectors in Saint Johns County. The trap types analyzed were the Biogents Sentinel Trap, Centers for Disease Control (CDC) Light trap, the Sentinel Mosquito Arbovirus Capture Kit, Mosquito Magnet X trap, CDC resting trap, and gravid traps. For the gravid traps, two different infusions were tested: hay infusion and cattail infusion. *Aedes atlanticus* Dyar and Knab was the most abundant EEEV vector captured in this study. Other EEEV vectors collected were *Aedes vexans* (Meigen), *Culex erraticus* (Dyar and Knab), and *Culex nigripalpus* Theobald. BG traps caught the highest abundance of EEEV vectors ( $1520 \pm 743$ ) compared to all the other trap types analyzed. Despite capturing multiple EEEV vectors during the testing period at the chosen site, *Culiseta melanura* (Coquillett) and *Coquillettidia perturbans* (Walker) were never captured.

Key Words. Eastern equine encephalitis, *Culiseta melanura*, *Coquillettidia perturbans*, *Aedes atlanticus*, Biogents sentinel traps

## INTRODUCTION

Eastern equine encephalitis virus (EEEV) causes a rare but serious disease in humans and is transmitted by the bite of infected mosquitoes (Nasci et al. 1996). Most human infections go unreported because they produce little to no illness, but in rare cases of EEE the infection results in either systemic or encephalitic infections. Systemic infections start quickly and present flu-like symptoms such as fatigue, fever, and muscle and joint pain. If the infection becomes neuroinvasive, a few days after presenting systemic symptoms the brain begins to swell resulting in continued fever, headaches, mood changes, coma, and convulsions. Most importantly, the fatality rate from encephalitic infections with EEE is approximately 33%, usually within 2-10 days of symptom onset. Those who recover from the disease are usually left with crippling

physical and mental disabilities for the remainder of their lives (CDC 2018). Eastern equine encephalitis virus is an alphavirus vectored primarily by *Culiseta melanura* in an enzootic cycle with passeriformes birds (Soghigian et al. 2018), but humans and horses act as incidental dead-end hosts. A majority of cases are reported in the Atlantic and Gulf coast states (i.e., Florida), but recently EEEV has been reported in more northern regions of the United States. In addition, *Cs. melanura* is suggested to act as a bridge vector to horses and humans in the northern part of the U.S. (Ibid). Other bridge vectors of EEEV that live in Florida include *Coquillettidia perturbans* (Andreadis et al. 1998; Bosak et al. 2001), *Aedes atlanticus* (Bigler 1976), *Culex nigripalpus* (Day and Stark 1996), *Culex erraticus* (Bingham et al. 2016), and *Aedes vexans* (Armstrong and Andreadis 2010). These bridge vectors are the mosquitoes that can potentially trans-

mit EEEV, along with other mosquito-borne arboviruses, to people (ibid).

Vector control is one of the options used to prevent infections of EEEV as there is no human vaccine for this rare but deadly pathogen. However, there is a horse vaccine that can prevent highly pathogenic infection of EEEV. In 2018, Florida detected EEEV in three humans, 52 horses, one mule, one donkey, one owl, two emus, 5 emu flocks, three mosquito pools, and 154 sentinel chickens across 33 counties (Morrison 2018). Eastern equine encephalitis has a mortality rate of >90% in horses with most survivors suffering permanent brain damage (ISU 2003). Optimized virus detection and trapping protocols for the collection of *Cs. melanura* and the bridge vectors of EEEV are vital for the protection of mosquito control clients.

There are multiple physiological states in mosquitoes of which trapping protocols can take advantage: host-seeking, gravid, and resting. Host-seeking traps utilize carbon dioxide and skin-odorant mimics, such as BG lure or octenol, to attract mosquitoes in search of a blood meal. Gravid traps utilize a fermented infusion water attractant, such as hay or alfalfa, to capture mosquitoes seeking oviposition sites. Finally, resting mosquitoes usually look for shady, cool, and moist areas to rest after active flight during the day or to digest a blood-meal. This study aims to find the trap or combination of traps that capture the greatest numbers and most diverse array of EEEV vectors in the different physiological states listed above to help guide viral surveillance and action threshold determination.

Six trap types were tested in the summer of 2017 for their attractiveness to EEEV vectors: Biogents Sentinel Traps (BG), Centers for Disease Control Light traps (CDC light), the Sentinel Mosquito Arbovirus Capture Kit (SMACK) (Johnson et al. 2015), Mosquito Magnet X trap (MMX), CDC resting trap (Panella et al. 2011), gravid trap with a hay infusion, and a gravid trap with a cattail infusion. Some EEEV vectors, such as *Cq. perturbans*, deposit eggs in permanent water sources rich in cattail plants, specifically *Typha latifolia* (Poirier et al. 2011). Fermented

cattail plants were used as an oviposition attractant in a gravid trap to bias its collection towards *Cq. perturbans*. Each trap was analyzed for abundance and mosquito diversity captured in a 24-hour period. Abundance is important for mosquito control districts to set action thresholds for operational efforts, and species diversity was assessed to make sure both primary and bridge vectors were collected for future mosquito pooling. Along with diversity and abundance analyses, rainfall, temperature, and other environmental data were collected for each trapping week to determine any weather anomalies that may have occurred during the study. We hypothesized that host-seeking traps would collect a higher number and greater diversity of EEEV vectors than the gravid and resting trap types due to the heavy attractiveness of the lures used. However, gravid and resting traps may capture more mosquitoes carrying virus even though their abundance may be lower because gravid and resting female mosquitoes most likely fed at least once. Also, we suspected the cattail infusion water would capture gravid *Cq. perturbans* while the hay infusion would attract other bridge vectors of EEEV. Finally, this study only assesses trap performance and not vector status or physiological state due to technical and cost limitations associated with viral assessment studies (i.e., RT-PCR and availability of cost-effective molecular kits).

## MATERIALS AND METHODS

The site used for this study was 1310 Saint Marks Pond, Saint Augustine, FL 32095 (29.979097, -81.386772) from mid-June to mid-July 2017. This site was selected for the experiment based on previous surveillance of multiple areas with a CDC light trap, which suggested that Saint Marks Pond had an abundance of *Cq. Perturbans*, as approximately 100 females were captured in a single night. Traps were placed at designated spots along a grass line that bordered dense vegetation (Fig. 1). There was a large horse ranch and farmland not far from the traps. BG traps (Biogents AG, Regensburg, Germany) utilized a 12-volt battery and a



Figure 1. Picture of trapping site and trap layout. The site used for this study was 1310 Saint Marks Pond, Saint Johns County, FL (29.979097, -81.386772). The 7 trap sites are marked with white flags within grey circles. The six trap types (with two infusion water types for the gravid traps) were rotated each trapping night between these 7 sites in a Latin shift design.

combination of dry ice and BG lure to attract host-seeking mosquitoes. CDC light traps (John Hock, Gainesville, FL) were baited with dry ice and hung from shepherd's hooks about 1 m above the ground. Mosquitoes captured by the CDC light trap

were contained in a jar spiked with no-pest kill strips. SMACK traps (Bioquip, Rancho Dominguez, CA) were hung from a shepherd's hook and a small 2 L cooler containing dry ice was hung inside the trap to lure mosquitoes inside the box. The screen

mesh that the mosquitoes crawl through to get in the SMACK trap is designed to form a one-way entrance for mosquitoes. MMX traps (American Biophysics Corp., RI) use a counter flow suction system to pull mosquitoes through the bottom of the trap into a capture chamber that keeps the mosquitoes alive. The trap is powered by a 12-volt car battery, and dry ice was used as the attractant to lure mosquitoes close enough to be pulled in by the suction. CDC Gravid traps (Bioquip, Rancho Dominguez, CA) were operated by 6-volt batteries with either a hay or cattail infusion in blue plastic tote containers. A black suction unit mounted on the tote container was covered with a fine white mesh capture bag for ease of collection of adult gravid mosquitoes. Two infusion water types were used to attract EEEV vectors: hay infusion and a cattail infusion. Both hay and cattail infusions used the same ratio of plant substrate to water: 24 g substrate in 8 Liters of water (Irish et al. 2013). These infusions fermented for one-week prior to testing, and were refreshed every week. Infusions were diluted 10% (v/v) in tap water and poured into the gravid trap receptacle. The CDC resting trap (Panella et al. 2011) consisted of a 35.56-cm diameter wood fiber pot modified with a black trash bag that covered the inner surface of the trap. An 8.89-cm diameter hole was cut into the bottom to house a vacuum apparatus from a CDC gravid trap. A fine white mesh capture bag was placed on the end of the suction unit for the capture of resting adult mosquitoes, and the trap was run on a 6-volt battery. Each trap was set 180 m apart and left in the field overnight to collect mosquitoes. Depending on the trap, mosquitoes were collected differently. For the BG trap, CDC light trap, and gravid traps, mosquitoes were collected in their respective bag or jar. For the SMACK trap, resting trap, and MMX trap, the mosquitoes were aspirated using a mechanical vacuum with an attached mesh bag. Traps were moved to a new spot and reset using a Latin shift experimental design according to Figure 1. All samples were kept at room temperature when transported back to the lab for iden-

tification. Mosquitoes that were identifiable were logged in data sheets for future analysis, while mosquitoes that were unidentifiable due to damage were not included.

Temperature, wind speed, and rainfall were monitored using Weather Underground (The Weather Company, Ann Arbor, MI) from a weather station in zip code 32095. This was the closest weather station to the trap site.

The trap data were analyzed via JMP statistical software (SAS Institute, Cary, NC). Specifically, the average abundance of EEEV vectors captured in each trap ( $\pm$  SEM) were compared. A Shapiro-Wilk goodness of fit test was used to determine if the data were non-normal and heteroscedastic. A Kruskal-Wallis test was used to test for statistical significance in the data set and a Tukey-Kramer HSD test determined if there was a statistical difference between trap types. The results of the Tukey Kramer HSD were represented with letters above each bar. Species diversity captured in each trap was analyzed using the Shannon Diversity Index (Rosenzweig 1995).

## RESULTS

*Environmental parameters.* Overall, the average lowest temperature over the entire trapping period was 23.6°C and the average highest temperature was 31.1°C. Wind speed ranged from 4.83-17.7 km/h. Rainfall varied between 0 and 12.7 cm. Most of the rainfall occurred within the first two weeks of the testing period. The total number of EEEV and non-EEEV vectors were calculated from each trapping day (Fig. 2). The highest abundance of mosquitoes was collected from July 11 to July 17, and our heaviest rainfall (12.7 cm in one week) occurred two weeks prior to that higher capture rate.

*Trap performance.* A total of 29,887 mosquitoes were captured over one month, and 19,532 of the trapped mosquitoes were EEEV vectors. In general, host-seeking traps collected more mosquitoes than the gravid and resting traps in terms of EEEV vector abundance, and there was no statistically significant difference between the capture

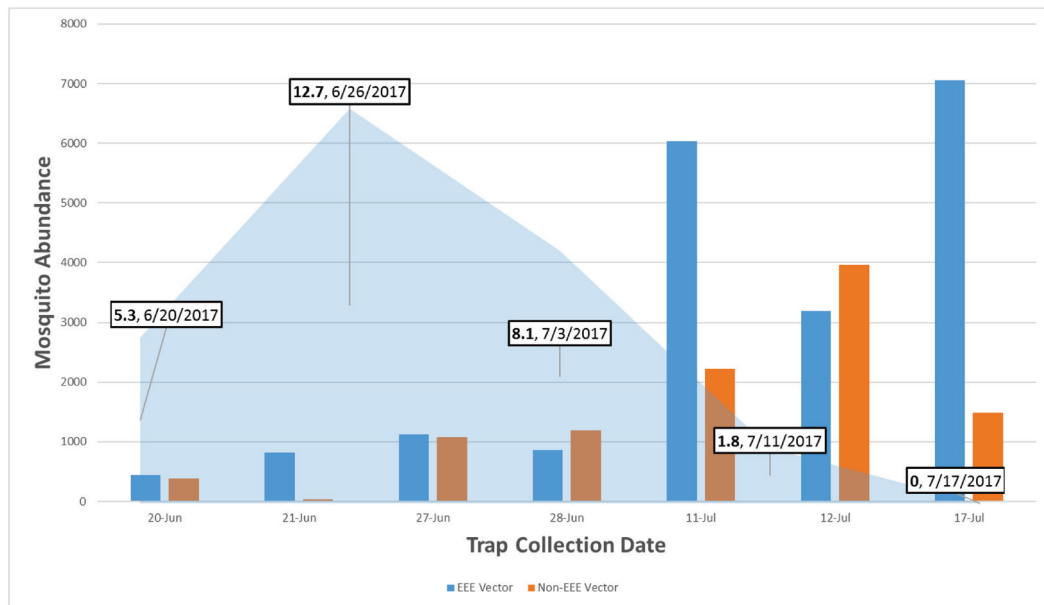


Figure 2. Total trap collections per day and rainfall patterns. In this graph, rainfall levels and total daily collections of EEEV and Non-EEEV vectors were analyzed. The x-axis indicates day of trap collections while the y-axis indicates total abundance of mosquitoes. The transparent area graph behind the bars show the pattern of rainfall during the trap duration. Rain was calculated for each week using Weather Underground precipitation data. The total rainfall (in centimeters) for that week is represented in a box next to the trap day for each week of the study. Blue bars represent EEEV vectors and orange bars represent Non-EEEV vectors.

rate with hay and cattail infusions. Out of the 6 trap types analyzed, BG traps caught the highest number of EEEV vectors (DF = 6, F ratio = 3.55,  $p < 0.05$ ) (Fig. 3). BG traps caught an average of  $1520 \pm 743$  EEEV vectors per trap night. The CDC light trap ( $749 \pm 331$ ) and the SMACK trap ( $310 \pm 129$ ) caught the next highest number of EEEV vectors per night. Of note, the CDC light trap caught EEEV vectors every night of trapping. *Aedes atlanticus* was the most abundant EEEV vector captured in this assay, making up 78.8% of the total collection. Other EEEV vectors collected in this assay included *Ae. vexans*, *Cx. erraticus*, and *Cx. nigripalpus*. The CDC light trap and MMX trap captured the highest diversity of mosquitoes according to the Shannon Diversity Index ( $H = 1.580$  and  $1.505$ , respectively) compared to all the other trap types (shown in Table 1). *Aedes atlanticus* and *Cx. nigripalpus* were captured by all the trap types, but *Ae. vexans* was only detected in the CDC light trap and BG trap (160 individuals and one individual, respectively). Although the collection of *Ae. vexans* in CDC light traps was low, it was still

consistent with data collected by mosquito surveillance teams across the US and internationally (Walters and Lavoipierre 1982; Kline 1999, Miller et al. 2002; Andreadis et al. 2004; Molaei and Andreadis 2006; Williams and Gingrich 2007). For example, in Williams and Gingrich (2007) *Ae. vexans* was collected in CDC light traps the most compared to the gravid and resting boxes in their analysis in New Jersey and Delaware. *Culex erraticus* went to every trap except the gravid trap with cattail infusion.

## DISCUSSION

In terms of total EEEV vector abundance, the BG trap outperformed the other host-seeking, gravid, and resting traps. Out of the six possible vectors of EEEV in Saint Johns County, only 4 species were accounted for with trapping in this assay. In terms of total EEEV vectors captured, host-seeking traps far outperformed gravid and resting-based traps. More specifically, among the host-seeking traps, BG traps collected more



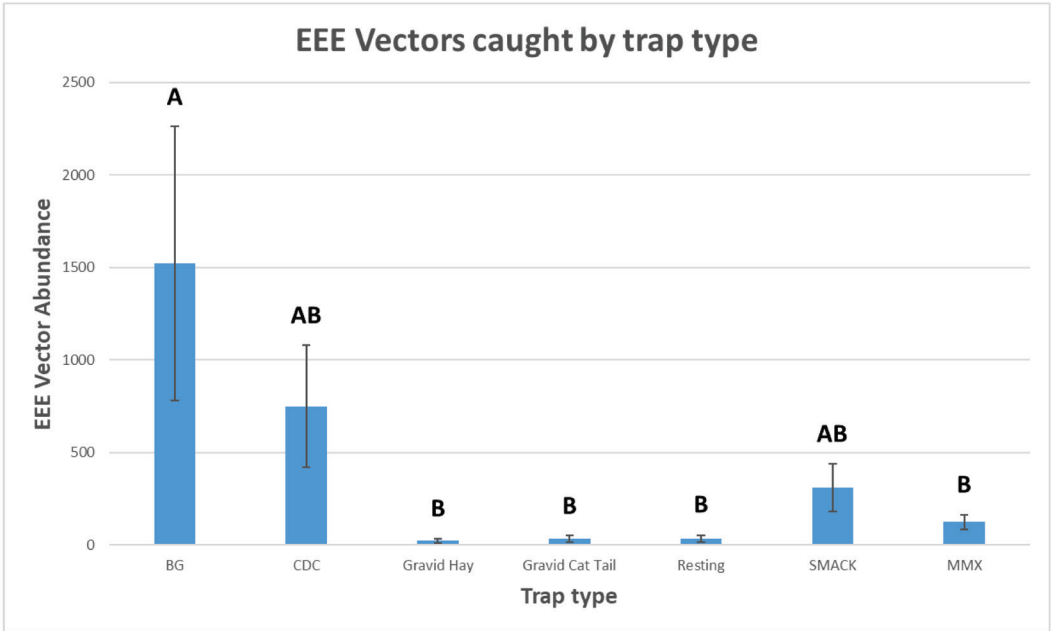


Figure 3. EEEV vectors caught by trap type. This graph shows the average ( $\pm$  SEM) daily collection of EEEV vectors by trap type. The x-axis represents trap type while the y-axis represents average daily EEEV vector abundance. The BG trap outperformed the other trap types ( $p \leq 0.05$ ). The Tukey-Kramer HSD is represented with letters above each bar, with different letters indicating statistical differences between trap types.

mosquitoes than the CDC light, SMACK and MMX traps.

The two most prominent vectors of EEEV in Saint Johns County are *Cs. melanura* and *Cq. perturbans*. Before setting traps in this area, a brief trapping survey was done to find sites with *Cs. melanura* and *Cq. perturbans*, and the Saint Mark’s Pond site was a hot-spot for *Cq. perturbans* but not *Cs. melanura* at the time. Despite not having any *Cs. melanura*, the site was used because of the high abundance of *Cq. perturbans* collected during the site survey. However, a serious limitation to this study was that neither *Cq.*

*perturbans* nor *Cs. melanura* were detected during the trapping period, which occurred shortly after the initial trapping survey was conducted. The likely cause for this absence of *Cq. perturbans* is its life history. *Coquillettidia perturbans* is considered a univoltine mosquito in Canada (Lewis and Bennett 1980; Allan et al. 1981), but has a multivoltine life cycle in Florida (Lounibos and Escher 1983). In addition, *Cq. perturbans* only has two to three brief periods of activity in which they are actively host seeking and/or gravid, but for a majority of the time they are in the larval and pupal stage (ibid). When the pre-

Table 1. Shannon diversity index of all mosquito species caught in each trap from the trapping area. The Shannon diversity index was calculated using the formula below, where  $s$  = number of species,  $\pi_i = n/N$ ,  $n$  = number of individuals of a species, and  $N$  = total number of individuals caught in each trap.

Trap	Number of species collected	Diversity index (H)
CDC light trap	11	1.580
MMX trap	9	1.505
BioGents Sentinel Trap	10	1.289
SMACK	8	1.232
Resting trap	8	0.833
Gravid trap with hay infusion	4	0.684
Gravid trap with cattail infusion	3	0.273

trapping work was done, that was likely during one of the peak periods of activity for *Cq. perturbans* adults. However, by the time the actual testing of the different host-seeking and gravid traps was conducted, *Cq. perturbans* activity may have been reduced because of adult die-off and the slow development time of the larvae. The absence of *Cs. melanura* is likely due to site selection. *Cs. melanura* prefer to breed near Cypress, and very little Cypress was observed at the site. Trapping assays for *Cs. melanura* can be improved through the selection of sites using historical data collected from CDC light traps. This would give research professionals a site to use for the testing of new and existing technologies for mosquito surveillance. Though the study was heavily limited by the absence of *Cq. perturbans* and *Cs. melanura*, this study did detect the presence of four other vectors of EEEV in Florida: *Ae. atlanticus*, *Ae. vexans*, *Cx. erraticus*, and *Cx. nigripalpus*, all of which could potentially transmit EEEV to Saint Johns County residents.

To increase the likelihood of finding EEEV infected vectors, capturing a higher proportion of mosquitoes that are either gravid or parous is ideal. Some of the different traps used in this assay may facilitate mosquito control personnel in that endeavor. BG traps, for example, capture a mixture of nulliparous, parous, and even gravid *Aedes* mosquitoes (Maciel-de-Freitas et al. 2006; Ball and Ritchie 2010; Barrera et al. 2013). Though BG traps do not favor parous or gravid mosquitoes, these traps still inform mosquito controls about the overall abundance of mosquitoes in the area. Gravid traps, as stated in their name, are more specific to gravid female mosquitoes, especially *Cx. quinquefasciatus* and *Cx. nigripalpus* (Day 2016). Different plant substrates used in the infusion can capture different mosquito species (ibid). Finally, resting traps can collect blood-fed mosquitoes, which is valuable for blood-meal analysis and vector disease status (Brown et al. 2018).

The resting box trap design was one of the trap types tested in the Saint Marks Pond area. Resting shelters were tested previously in New York to determine the abun-

dance of *Cs. melanura* captured at different time points (Howard et al. 2011). The resting shelters tested by Howard et al. (2011) caught an abundant amount of EEEV vectors when collected between 0900 and 1300 hours, but the design of these traps differs from the design that was used in this study. The CDC resting trap used in this assay primarily captures *Culex* and *Aedes* species (Panella et al. 2011), and it caught *Ae. atlanticus*, *Cx. erraticus*, and *Cx. nigripalpus* at the Saint Marks Pond site. One of the objectives of this study was to determine if the CDC resting trap would capture *Cs. melanura* in Florida, but this species was never trapped in the Saint Mark's Pond area during the time of this assay. Resting shelters used by Howard et al. (2011) are all black rectangular boxes designed by Morris (1981) that were modified with a lid lip made of fir stripping. The CDC resting trap utilizes a brown fiber pot and vacuum system. This system was used because it was potentially more efficient at capturing EEEV vectors due to the continuous suction of mosquitoes from the fan. However, it was not known if this model was applicable to *Cs. melanura* in Florida. Future work with resting shelters should include a direct comparison of the black resting shelter and the CDC resting trap for the capture of blood-engorged *Cs. melanura*.

Gravid traps are the preferred trap to use for capturing virus-infected mosquitoes because they target mosquitoes that have consumed a blood meal. For this study, depending on the infusion used, gravid traps caught as low as 20 and as high as 120 EEEV vectors. The hay infusion caught a total of 148 individuals while the cattail infusion caught a total of 234 individuals (data not shown). Additional investigations should be carried out using different gravid trap infusions and formulations over the course of an entire season to optimize this trap type for the collection of mosquitoes that vector EEEV.

BG traps performed dependably and caught the most vectors. Despite the fact that BG traps collected a larger pool of mosquitoes, searching for gravid, blood-fed, and parous mosquitoes amid hundreds of nulliparous unfed mosquitoes can be tedious.

Interestingly, a recent publication suggested modifications to the BG trap that keep mosquitoes alive for much longer and allow for the incorporation of FTA cards for arbovirus detection (Timmins et al. 2018). This modification and associated FTA card incorporation should be tested alongside other host-seeking traps (such as the SMACK and MMX traps) for the detection of EEEV from bridge vectors.

To conclude, this study succeeded in comparing the existing toolset available to mosquito control districts for the surveillance of EEEV vectors. BG traps were shown to collect numerous EEEV vectors comparable to that of the standard CDC light trap. Having a highly sensitive trap like the BG trap that can capture numerous EEEV vector mosquitoes is advantageous to mosquito control districts because action thresholds can vary depending on the number of EEEV cases in an area. Sometimes, action thresholds are lowered substantially, especially during an outbreak of EEE. The collection of rare or low-abundance vectors at critical times is important to mosquito control districts because it allows them to find the vector quickly and treat the area as soon as possible. Accurate and up-to-date surveillance tools are necessary for the prevention of disease outbreaks in Saint Johns County, FL. This study, and continued efforts like this, will facilitate mosquito abatement districts by giving them information about the current tools in their arsenal and how to use those tools to find new disease threats around their constituents.

#### REFERENCES CITED

- Allan SA, Surgeoner GA, Helson BV, Pengelly DH. 1981. Seasonal Activity of *Mansonia perturbans* adults (Diptera: Culicidae) in southwestern Ontario. *Can Entomol* 113(2):133-139.
- Andreadis TG, Anderson JF, Tirrell-Peck SJ. 1998. Multiple isolations of eastern equine encephalitis and highlands J viruses from mosquitoes (Diptera: Culicidae) during a 1996 epizootic in southeastern Connecticut. *J Med Entomol* 35(3):296-302.
- Andreadis TG, Anderson JF, Vossbrinck CR, Main AJ. 2004. Epidemiology of West Nile virus in Connecticut: a five-year analysis of mosquito data 1999-2003. *Vector Borne Zoonotic Dis* 4(4):360-378.
- Armstrong PM, Andreadis TG. 2010. Eastern equine encephalitis virus in mosquitoes and their role as bridge vectors. *Emerg Infect Dis* 16(12):1869-1874.
- Ball TS, Ritchie SR. 2010. Sampling biases of the BG-sentinel trap with respect to physiology, age, and body size of adult *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 47(4):649-656.
- Barrera R, Mackay AJ, Amador M. 2013. An improved trap to capture adult container-inhabiting mosquitoes. *J Am Mosq Control Assoc* 29(4):358-368.
- Bigler WJ, Lassing EB, Buff EE, Prather EC, Beck EC, Hoff GL. 1976. Endemic eastern equine encephalomyelitis in Florida: a twenty-year analysis, 1955-1974. *Am J Trop Med Hyg* 25(6):884-890.
- Bingham AM, Burkett-Cadena ND, Hassan HK, Unnasch TR. 2016. Vector competence and capacity of *Culex erraticus* (Diptera: Culicidae) for eastern equine encephalitis virus in the southeastern United States. *J Med Entomol* 53(2):473-476.
- Bosak PJ, Reed LM, Crans WJ. 2001. Habitat preference of host-seeking *Coquillettidia perturbans* (Walker) in relation to birds and eastern equine encephalomyelitis virus in New Jersey. *J Vector Ecol* 26(1):103-109.
- Brown R, Hing CT, Formace K, Ferguson HM. 2018. Evaluation of resting traps to examine the behaviour and ecology of mosquito vectors in an area of rapidly changing land use in Sabah, Malaysian Borneo. *Parasit Vectors*, 11(1):346.
- Centers for Disease Control. 2018. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). [Internet] Atlanta, GA: Eastern Equine Encephalitis - Symptoms and Treatment [accessed June 7, 2019]. <https://www.cdc.gov/easternequineencephalitis/tech/symptoms.html>
- Day JF. 2016. Mosquito Oviposition Behavior and Vector Control. *Insects*, 7(4):65.
- Day JF, Stark LM. 1996. Eastern equine encephalitis transmission to emus (*Dromaius novaehollandiae*) in Volusia County, Florida: 1992 through 1994. *J Am Mosq Control Assoc*, 12(3 Pt 1):429-436.
- Howard JJ, Oliver J, Kramer LD. 2011. Assessing the use of diurnal resting shelters by *Culiseta melanura* (Diptera: Culicidae). *J Med Entomol* 48(4):909-913.
- Irish SR, Moore SJ, Derua YA, Bruce J, Cameron MM. 2013. Evaluation of gravid traps for the collection of *Culex quinquefasciatus*, a vector of lymphatic filariasis in Tanzania. *Trans R Soc Trop Med Hyg* 107(1):15-22.
- Johnson BJ, Kerlin T, Hall-Mendelin S, van den Hurk AF, Cortis G, Doggett SL, Ritchie SA. 2015. Development and field evaluation of the sentinel mosquito arbovirus capture kit (SMACK). *Parasit Vectors* 8(1):509.
- Kline DL. 1999. Comparison of two American Biophysics mosquito traps: the professional and a new counterflow geometry trap. *J Am Mosq Control Assoc* 15(3):276-282.
- Lewis DJ, Bennett GF. 1980. Observations on the biology of *Mansonia perturbans* (Walker) (Diptera: Culicidae) in the Nova Scotia-New Brunswick border region. *Can J Zool* 58(11):2084-2088.
- Lounibos LP, Escher RL. 1983. Seasonality and sampling of *Coquillettidia perturbans* (Diptera: Culicidae) in South Florida. *Environ Entomol* 12(4):1087-1093.
- Maciel-de-Freitas R, Eiras AE, Lourenço-de-Oliveira R. 2006. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult *Aedes*

- aegypti* (Diptera: Culicidae). *Mem Inst Oswaldo Cruz* 101(3):321-325.
- Miller BR, Godsey MS, Crabtree MB, Savage HM, Al-Mazrao Y, Al-Jeffri MH, Ksiazek TG. 2002. Isolation and genetic characterization of Rift Valley fever virus from *Aedes vexans arabiensis*, Kingdom of Saudi Arabia. *Emerg Infect Dis* 8(12):1492-1494.
- Molaei G, Andreadis TG. 2006. Identification of avian- and mammalian-derived bloodmeals in *Aedes vexans* and *Culiseta melanura* (Diptera: Culicidae) and its implication for West Nile virus transmission in Connecticut, U.S.A. *J Med Entomol* 43(5):1088-1093.
- Morris CD. 1981. A structural and operational analysis of diurnal resting shelters for mosquitoes (Diptera: Culicidae). *J Med Entomol* 18(5):419-424.
- Morrison A. 2018. Florida Department of Health, Division of Disease Control and Health Protection. [Internet] Tallahassee, FL: Mosquito-Borne Disease Surveillance, Week 45 [accessed June 7, 2019]. <http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html>
- Nasci RS, Berry RL, Restifo RA, Moore CG. 1996. Population size, parity structure, and wing length of *Coquillettidia perturbans* in an Ohio focus of eastern equine encephalitis. *J Am Mosq Control Assoc* 12(1):64-68.
- ISU. 2003. Center for Food Security and Public Health. Iowa State University College of Veterinary Medicine. Eastern equine encephalomyelitis, western equine encephalomyelitis, and Venezuelan equine encephalomyelitis. [Internet]. Ames, IA: [Accessed June 7, 2019]. [https://www.nj.gov/agriculture/divisions/ah/diseases/equine\\_encephalomyelitis.html](https://www.nj.gov/agriculture/divisions/ah/diseases/equine_encephalomyelitis.html)
- Panella NA, Crockett RJK, Biggerstaff BJ, Komar N. 2011. The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. *J Am Mosq Control Assoc* 27(3):323-325.
- Poirier LM, Berry KE. 2011. New distribution information for *Coquillettidia perturbans* (Walker) (Diptera, Culicidae) in northern British Columbia, Canada. *J Vector Ecol* 36(2):461-463.
- Rosensweig M. 1995. *Species Diversity in Space and Time*. University of Arizona: Cambridge University Press.
- Soghigian J, Andreadis TG, Molaei G. 2018. Population genomics of *Culiseta melanura*, the principal vector of Eastern equine encephalitis virus in the United States. *PLOS Neglected Tropical Diseases* 12(8):e0006698.
- Timmins DR, Staunton KM, Meyer DB, Townsend M, Paton CJ, Ramírez AL, Ritchie SA. 2018. Modifying the Biogents Sentinel trap to increase the longevity of captured *Aedes aegypti*. *J Med Entomol* 55(6):1638-1641.
- Walters L, Lavoipierre M. 1982. *Aedes vexans* and *Aedes sierrensis* (Diptera, Culicidae) - potential vectors of *Dirofilaria immitis* in Tehama County, northern California, USA. *J Med Entomol* 19(1):15-23.
- Williams G, Gingrich J. 2007. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. *J Vector Ecol* 32(2):285-291.

**CONTROL OF ADULT AND LARVAL *Aedes albopictus*  
WITH ATTRACTIVE TOXIC SUGAR BAITS  
(ACTIVE INGREDIENT: CINNAMON-SESAME OIL)  
IN NORTHEASTERN FLORIDA**

MOHAMED M. TRAORE<sup>1</sup>, AMY JUNNILA<sup>1</sup>, EDITA E. REVAY<sup>2</sup>,  
VASILIY D. KRAVCHENKO<sup>3</sup>, ARLENE LAHTI<sup>4</sup>, JODY M. FIOREZZANO<sup>5</sup>,  
WHINTEY A. QUALLS<sup>6</sup>, DANIEL L. KLINE<sup>7</sup>, YOSEF SCHLEIN<sup>8</sup>,  
JOHN C. BEIER<sup>9</sup>, RUI-DE XUE<sup>5</sup>, AND GUNTER C. MULLER<sup>1,8,\*</sup>

<sup>1</sup>Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Odontostomatology, University of Sciences, Techniques and Technology of Bamako, Bamako, Mali, BP 1805, Bamako, Mali

<sup>2</sup>Department of Anatomy and Cell Biology, Bruce Rappaport Faculty of Medicine, Technion, Haifa 34995, Israel

<sup>3</sup>Department of Zoology, Tel Aviv University, Tel Aviv, Israel

<sup>4</sup>Northwestern Scientific, Thunder Bay, ON, P7G 0K9, Canada

<sup>5</sup>Anastasia Mosquito Control District, 120 EOC Dr., St. Augustine, FL 32092, U.S.A.

<sup>6</sup>Texas Department of State Health Services/Zoonosis Control Branch, 1100 West 49th Street, Austin, TX 78714, U.S.A.

<sup>7</sup>U.S. Department of Agriculture – Agricultural Research Service, 1600-1700 SW 23rd Drive, Gainesville, FL 32608, U.S.A.

<sup>8</sup>Department of Microbiology and Molecular Genetics, IMRIC, Kuvim Centre for the Study of Infectious and Tropical Diseases, Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel

<sup>9</sup>Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, Florida, 33136, U.S.A.

\*To whom correspondence should be addressed: [guntercmuller@hotmail.com](mailto:guntercmuller@hotmail.com)

Subject Editor: Eva Buckner

ABSTRACT

Because traditional methods of mosquito control using insecticides has produced resistance, new methods that are environmentally friendly, sustainable and cost effective have been sought. One method, attractive toxic sugar baits (ATSB), uses the biological requirements, ecology, and behavior of mosquitoes to attract and kill them. In this study, the efficacy of a new ATSB active ingredient, microencapsulated cinnamon oil-plus-sesame oil, was tested in the laboratory and field against *Aedes albopictus* (Skuse) and the effect on non-target organisms was evaluated. The average mortality among groups of 20 third instar larvae after exposure to microencapsulated cinnamon-sesame oil ATSB in the laboratory for 48 h was high. Mortality at 10% and 1% ATSB concentration was 95.8% and 90.0% respectively and began to drop off (to 65%) at 0.1%. After application of the ATSB in the field, on day 11 of the

study, the adult *Ae. albopictus* populations at the experimental site dropped significantly compared to pre-treatment levels and to the untreated control population. The differences between the control and the treated sites remained significant until the end of the study period on day 28. If used in accordance with label instructions and applied on non-flowering green vegetation, the potential impact on non-target populations was negligible with the exception of non-biting midges (Chironomidae). The synergistic effect of the attracting and killing adult mosquitoes as well as wash-off into part of the breeding sites with larvicidal cinnamon oil-plus-sesame oil product likely explains the high mortality of this ATSB formulation.

Key Words: Attractive Toxic Sugar Baits (ATSB); *Aedes albopictus*; cinnamon oil; sesame oil; larvicide; adulticide

---

## INTRODUCTION

*Aedes* (Stegomyia) mosquitoes, found over several continents across the globe, are of great public health importance as they are vectors for many pathogens, including Zika virus. *Aedes aegypti* (Linnaeus) and *Ae. albopictus* have been shown to transmit the Zika virus (Hayes 2009, CDC 2016). They are a major concern due to their widespread distribution throughout the tropical and subtropical world. In recent years, these species have been introduced to new areas, like the Americas and Europe (Marcondes and Ximenes 2016). *Aedes albopictus* can exist in more temperate areas than *Ae. aegypti*, thus extending the potential range where outbreaks may occur. In the United States, *Ae. aegypti* is endemic throughout Puerto Rico, the U.S. Virgin Islands, Hawaii, and in parts of the contiguous United States (Monaghan et al. 2016). As climate change continues to alter the environment, these mosquitoes may spread further and consequently, the diseases they carry may spread as well.

Because reliance on a single chemical class of insecticides can lead to resistance in mosquito populations and may compromise future control efforts, new control methods that are environmentally friendly, sustainable and cost-effective have been sought. These new methods can be used in combination with conventional insecticides or alone. One method uses the biological requirements, ecology, and behavior of mosquitoes to attract and kill them. Called attractive toxic sugar baits (ATSB), the method uses the mosquito need for a sugar meal shortly after emergence and throughout their lives (Schlein and Muller 2008). Sugar-feeding female and male mosquitoes attracted to ATSB formulations, either sprayed on plants

or in bait stations, ingest an incorporated low-risk toxin and are killed (Beier et al. 2012). ATSB methods have been extensively tested throughout the last few years and are highly effective in controlling mosquitoes (Fiorenzano et al. 2017).

ATSB has been tested with several different active ingredients, such as boric acid sugar bait at 1% W/V (Xue and Barnard 2003, Beier et al. 2012; Qualls et al. 2015; Wang et al. 2017), dinotefuran (Khallaayoune et al. 2013), eugenol (Revay et al. 2014; Qualls et al. 2014), pyriproxyfen (Fulcher et al. 2014), and spinosad (Müller and Schlein 2008; Müller et al. 2008; Müller et al. 2010) and microencapsulated garlic oil (Junnala et al. 2015). The goals of this study were to test the efficacy of a new ATSB active ingredient, microencapsulated cinnamon oil-plus-sesame oil, in the laboratory and field against *Ae. albopictus*, and to evaluate the potential impact of the cinnamon-sesame oil formulation on non-target organisms. United States Environmental Protection Agency (EPA) guidelines for field testing insecticides were closely followed (EPA 712-C-017).

## MATERIALS AND METHODS

**Laboratory experiments.** The impact of a ready to use ATSB cinnamon oil-sesame oil formulation (Westham Innovations Ltd., Tel Aviv, Israel) on *Ae. albopictus* larvae was tested according to standard guidelines for testing larvicidal products (Debboun et al. 2006). The ATSB concentrate was diluted 1:3 with tap water. Larvae were supplied by the United States Department of Agriculture (USDA) Gainesville, FL, USA. Tests were conducted in the laboratory of the Anastasia Mosquito Control District, FL, USA. Six ATSB concentrations (10.0, 1.0, 0.1, 0.01,

0.001 and 0.0001%) were prepared in 500 ml laboratory beakers of ATSB and tested against six cohorts of 20 third instar mosquito larvae. Mortality was recorded 48 h after exposure. Untreated controls and experimental cohorts were kept under standard insectary conditions.

**Experimental sites and conditions.** Field experiments were conducted in northeastern Florida in suburban/rural St. Augustine, from early November to early December, at three sites: a control site, an experimental site, and a site for monitoring non-target organism impact. The control site was a residential area at 29° 93' 04.9" N, 81° 34' 38.76" W, on the outskirts of St. Augustine surrounded by parkland, pine forests and wetlands. This site did not receive any treatment. Mosquito monitoring traps were placed in a yard with numerous flooded containers, refuse, and extensive bamboo thickets.

The experimental site was a farm/junkyard in an agricultural area, at 29° 46' 44.2" N, 81° 28' 08.5" W, Elkton, Florida, U.S.A., surrounded by open fields and irrigation ditches. The area covered about 1.6 ha (4 acres) and had an abundance of farm junk, including about one hundred tires. A portion of the tires and the farm junk were naturally filled with water and offered suitable breeding sites for *Ae. albopictus*. A non-target organism monitoring site, where the impact of ATSB on organisms such as butterflies and bees was measured, was located near the property of the Anastasia Mosquito Control District headquarters, and consisted of open wasteland, retention ponds, and the edges of a pine and oak forest. During the study period, it was exceptionally warm and mild with unusually high mosquito populations.

**Bait application.** At the experimental site, the area was treated with an ATSB formulation containing microencapsulated cinnamon oil-sesame oil as the active ingredient (Westham Innovations Ltd., Tel Aviv, Israel). Treatment was applied according to manufacturer's instructions. Briefly, ATSB was applied to non-flowering vegetation, covering about 5% of the total area and was sprayed to wet the vegetation until just before the point of run-off. The mixture was applied

with an All-terrain Vehicle (ATV) mounted spray apparatus, supplied by the Anastasia Mosquito Control District. The driver was moving at 8 km/h (5 miles/h) while a technician sprayed the vegetation, moving the nozzle up and down, to cover both the under and upper sides of the foliage.

At the non-target site, food dye-stained Attractive Sugar Bait (ASB; Westham Innovations Ltd., Tel Aviv, Israel) was applied by backpack sprayer (Hozelock, Birmingham, UK), according to manufacturer's instructions, to evaluate potential impact on non-target organisms. It has been shown that ATSB may induce behavioral changes in target and non-target insects before killing them (Qualls et al. 2015), therefore, trials with ATSB would yield falsely low results as only a fraction of the poisoned insects could be recovered. By using non-toxic Attractive Sugar Bait (ASB), and verifying color presence in the insect gut, the highest possible exposure of non-targets to the bait can be measured. At this site, three plots of 1000 m<sup>2</sup>, relatively rich in flowers for late in the season, were selected. Plots were 500m apart from each other. Flowering vegetation comprised about 3% of the area; the green vegetation comprised the rest of the area.

The ASB was prepared from a concentrate by diluting it 1:3 with regular tap water and adding, if applied on flowering vegetation 0.5% yellow food dye and if applied on non-flowering, green vegetation 0.5% green food dye. ASB was applied to the control site just as ATSB was applied to the experimental site using an ATV-mounted spray system.

**Monitoring.** At the experimental and control sites, mosquitoes were monitored before ATSB treatment and 2 to 3 times per week after treatment for the following 4 weeks (see Fig. 2). At both areas, six BG Sentinel Traps (BioQuip Products, Rancho Dominguez, CA, USA) were placed at least 25 m apart and baited with BG-Lures. The traps were monitored as described above.

Monitoring non-targets occurred with a large Malaise trap (6 m model 3012, John W. Hock, Gainesville FL, USA), six ultra violet-equipped Center for Disease Control (UV-CDC) traps (model 512, John W.

Hock, Gainesville FL, USA), two UV-tray traps (constructed according to Qualls et al. 2015), yellow-plates (yellow disposable plastic dinner plates, filled to the rim with isopropanol), and by collecting larger day-active insects with entomological hand nets and sweep nets. Non-targets were monitored in a separate trial for 7 days by placing one of several trap types in the center of each of the three 1000 m<sup>2</sup> plots.

Additionally, all three sites were visited for 30 min during three sunny days for collection with entomological hand nets and sweep nets. Feeding was verified by checking the gut content of random insect samples (Table 1) for stained bait. Any amount of feeding was regarded as a potential lethal dose.

**Statistics.** Laboratory results were compared using one-way ANOVA with a Dunnett's multiple comparison's test. Comparison of mean trap catches at treated and control sites were analysed by the t-test for each time point. Analysis was conducted using GraphPad Prism 7.00 for windows (GraphPad Software, La Jolla California, USA).

## RESULTS

**Weather conditions.** Weather remained relatively constant throughout the study period. Light rain (< 1 cm) occurred on days 1, 8, 9, 11, 12, 13, 17, 18, 19, 22 and 25. Heavy rain occurred on day 21. The highest temperature was 31.7°C (89°F) and the lowest temperature was 16°C (62°F).

**Laboratory experiments.** Results of laboratory experiments are shown in Fig. 1. Larval mortality at 10% and 1% was high with 18.0±0.7 and 19.1±0.5 dead larvae (95.8% and 90.0% larval mortality respectively). At 0.1%, the larvicidal effect begins to decrease to an average of 13.0 dead larvae (65%). Each concentration, except 0.0001% (P = 2.99) was significantly different than the control (P > 0.05).

**Field experiments. ATSB trials with mosquitoes at experimental sites.** Results of the field experiments are shown in Fig. 2. After application of the ATSB formulation, on day

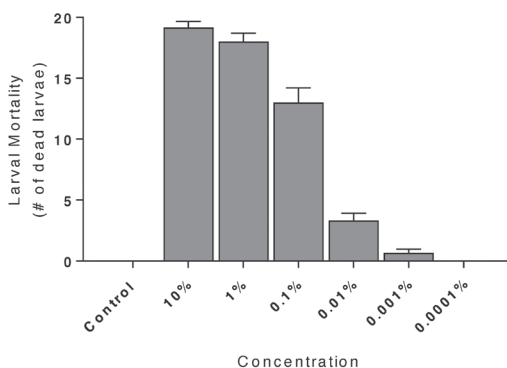


Figure 1. Average mortality among groups of 20 3<sup>rd</sup> instar larvae after exposure to microencapsulated cinnamon-sesame oil for 48 h. Experiments were repeated 6 times.

8 of the study, the *Ae. albopictus* populations at the experimental site dropped significantly compared to pre-treatment levels (P < 0.5; t=4.78, df =9) and to the untreated control population. The differences between the control and the treated sites remained significant until the end of the study period on day 28 (P < 0.5; t=7.22, df =9).

**ASB trials on non-target organisms.** There is no apparent difference between feeding rates of mosquitoes if ASB is sprayed on non-flowering or on flowering vegetation (Table 1). If used in accordance with label instructions and applied on non-flowering green vegetation the potential impact on non-target populations is negligible with the exception of non-biting midges (Chironomidae). If improperly applied on flowering vegetation, the impact on non-targets can be high, though honey bees are not attracted and seemed to avoid the bait even on flowering vegetation (Traore et al. unpublished data).

## DISCUSSION

Efforts to eliminate the mosquito breeding habitat depend on larvicides yet they are also susceptible to some traditional difficulties such as locating and delivering the larvicide to the breeding sites effectively and the emergence of resistance (Chandre et al. 1998; Chaki et al. 2009). New larvicidal compounds with the potential to be delivered to breeding sites in the form of run-off could



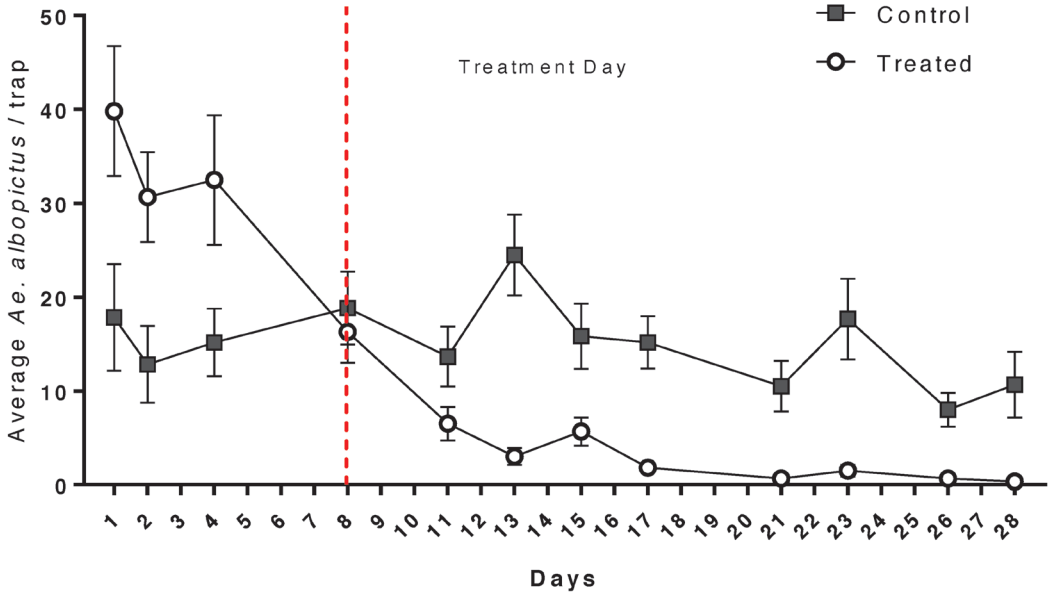


Figure 2. Average BG trap catches of *Ae. albopictus* after ATSB treatment of an experimental site compared to an untreated control site.

be useful. In this study, it was demonstrated that a new ATSB formulation with cinnamon oil-sesame oil as the active ingredient demonstrated significant larvicidal activity on *Ae. albopictus* in laboratory trials at concentrations as low as 0.1%. It has been previously shown that cinnamon oil and its components are larvicidal to several mosquito species including *Ae. albopictus* (Zhu et al. 2006, Zhu et al. 2008).

There is plenty of concern among consumers and the broader public about the safety and long-term effects of insecticides. It is reasonable to assume that in open, large water bodies, ATSB becomes too diluted to cause mortality of both nontargets and mosquitoes. In artificial containers, there are no non-targets that are of any environmental concern, and small amounts of the bait sprayed purposely, by drift, or wash off by rain can reach high enough concentrations

Table 1. Target and non-target organism staining after ASB treatment of green vegetation (about 5% of total vegetation) and flowering vegetation (about 3% of total). Orders are indicated in bold.

	Green vegetation			Flowering vegetation	
	# Examined	#ASB Positive	% Positive ASB	# ASB Positive	% Positive ASB
<b>TARGETS</b>					
Mosquitoes	400	93	23.25%	107	26.75%
<b>NON-TARGETS</b>					
Coleoptera	500	4	0.80%	18	3.60%
Higher Diptera	1000	17	1.70%	162	16.20%
Chironomidae	1000	190	19.00%	287	28.70%
Hemiptera	100	0	0.00%	11	11.00%
Hymenoptera	500	3	0.60%	97	19.40%
Honey-bees	200	0	0.00%	2	1.00%
Lepidoptera	1000	6	0.60%	105	10.50%
Orthoptera	250	2	0.80%	14	5.60%

to result in significant mosquito mortality. In the current study, the ATSB formulation attracted and killed significant numbers of adult *Ae. albopictus* while non-toxic ASB attracted and marked very few non-target insects, especially pollinators when applied in accordance with label instructions ie: to not spray flowering plants in particular, which is in agreement with previous studies (Qualls et al. 2014, Qualls et al. 2015, Revay et al. 2015; Fiorenzano et al. 2017). We did notice a high number of Chironomidae feeding on the ASB and so ATSB might have potential to control swarming nuisance flies.

The excellent performance of this new ATSB mixture may be attributed to the larvicidal properties of the cinnamon oil-sesame oil formulation as the active ingredient. During the application, sprayed ATSB product droplets probably landed in some breeding sites and consecutive days of light rains washed the larvicidal product off the upper leaf surfaces and into the breeding sites. The synergistic of attracting and killing adult mosquitoes as well as contaminating at least part of the breeding sites with larvicidal product likely explains the better than expected performance of the tested ATSB formulation.

The cinnamon-sesame oil formulation has a fairly pleasant odor that will not disturb residential users or contaminate applicator clothing and equipment. It also demonstrated the superior dual performance with characteristics of a larvicide (Traore et al. unpublished data) and adulticide. It is crucial to understand that the bait attractant and preservative are key to this new control method. In the past, several home-made were tested for effectiveness against mosquitoes (Müller and Schlein 2008) and it is important to note that results may vary greatly if proper protocols are not followed. The new commercially produced ATSB spray used here avoids the problem of inconsistency in bait formulation.

#### ACKNOWLEDGEMENTS

The authors wish to thank Mike Smith for his assistance in conducting this study

and the residents and property owners that allowed us to conduct the experiments on their properties.

#### REFERENCES CITED

- Beier JC, Müller GC, Gu W, Arheart KL, Schlein Y. 2012. Attractive toxic sugar bait (ATSB) methods decimate populations of *Anopheles* malaria vectors in arid environments regardless of the local availability of favoured sugar-source blossoms. *Malaria J* 11:31-37.
- CDC. Zika virus. Atlanta, GA: US Department of Health and Human Services, CDC. 2016. Available from: <http://www.cdc.gov/zika/index.html>
- Chaki PP, Govella NJ, Shoo B, Hemed A, Tanner M, Fillinger U, Killean GF. 2009. Achieving high coverage of larval-stage mosquito surveillance: challenges for a community-based mosquito control programme in urban Dar es Salaam, Tanzania. *Malaria J* 8:311-321.
- Chandre F, Darriet F, Darder M, Cuany A, Doannio JM, Pasteur N, Guillet P. 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol* 12:359-66.
- Debboun, M., Frances, S.P., Strickman, D. 2006. *Insect repellents: principles, methods, and uses*. Boca Raton, London, New York. CRC Press, Taylor and Francis Group.
- Dick GW, Kitchen SF, Haddow AJ. 1952. Zika virus (I): Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 46:509-520.
- Duffy MR, Chen TH, Hancock T, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbauer S, Dubray C, Guillaumot L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB, 2009. Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 360:2536-2543.
- EPA 712-C-017: Ecological Effects Test Guidelines 2012. OCSPP 850.3040: Field Testing for Pollinators.
- Fauci AS, Morens, DM. 2016. Zika Virus in the Americas – Yet Another Arbovirus Threat. *N Engl J Med* 374:601-604.
- Fiorenzano JM, Koehler PG, Xue RD. 2017. Attractive Toxic Sugar Bait (ATSB) For Control of Mosquitoes and Its Impact on Non-Target Organisms: A Review. *Int J Environ Res Public Health* 14:398.
- Foster WA. 1995. Mosquito sugar feeding and reproductive energetics. *Annu Rev Entomol* 40:443-474.
- Fulcher A, Scott JM, Qualls WA, Müller GC, Xue RD. 2014. Attractive toxic sugar baits mixed with pyriproxyfen sprayed on plants against adult and larval *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 51:896-899.
- Hayes EB. 2009. Zika virus outside Africa. *Emerg Infect Dis* 15: 1347-1350.
- Hennessey M, Fischer M, Staples JE. 2016. Zika virus spreads to new areas – region of the Americas, May 2015-January 2016. *Am J Transplant* 16:1031-1034.
- Junnilla A, Revay EE, Müller GC, Kravchenko VD, Qualls WA, Xue RD, Allan SA, Beier JC, Schlein Y. 2015. Efficacy of Attractive Toxic Sugar Baits (ATSB) against *Aedes albopictus* with garlic oil encapsulated in beta-Cyclodextrin as the active ingredient. *Acta Trop* 152:195-200.
- Khallaayoune K, Qualls WA, Revay EE, Allan SA, Arheart KA, Kravchenko VD, Xue RD, Schlein Y,

- Beier JC, Müller GC. 2013. Attractive toxic sugar baits: control of mosquitoes with the low risk active ingredient dinotefuran and potential impacts on non-target organisms in Morocco. *Environ Entomol* 42:1040-1045.
- MacNamara FN. 1954. Zika virus: a report on three human cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 48:139-145.
- Marcondes CB, Ximenes MF. 2016. Zika virus in Brazil and the danger of infestation by *Aedes (Stegomyia)* mosquitoes. *Rev Soc Bras Med Trop* 49:4-10.
- Monaghan AJ, Morin CW, Steinhoff DF, Wilhelmi O, Hayden M, Quattrochi DA, Reiskind M, Lloyd AL, Smith K, Schmidt CA, Scalf PE, Ernst K. 2016. On the seasonal occurrence and abundance of the Zika virus vector mosquito *Aedes Aegypti* in the contiguous United States. *PLOS Curr* [internet] 16. Edition 1. doi: 10.1371/currents.outbreaks.50dfc7f46798675fc63e7d7da563da76. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807952/>
- Müller GC, Schlein Y. 2008. Efficacy of toxic sugar baits against adult cistern dwelling *Anopheles claviger*. *Trans R Soc Trop Med Hyg* 102:480-484.
- Müller GC, Kravchenko VD, Schlein Y. 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive, toxic (Spinosad), sugar bait stations in an oasis. *J Am Mosq Control Assoc* 12:147-149.
- Müller GC, Junnilla A, Qualls W, Revay EE, Kline DL, Allan S, Schlein Y, Xue RD. 2010. Control of *Culex quinquefasciatus* in a storm drain system in Florida with attractive toxic sugar baits (ATSB). *Med Vet Entomol* 24:346-351.
- Müller GC, Junnilla A, Schlein Y. 2010. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval habitats. *J Med Entomol* 47:63-66.
- Qualls AW, Xue RD, Revay EE, Allan S, Müller GC. 2012. Implications for operational control of adult mosquito production in cisterns and wells in St. Augustine, FL using attractive sugar baits. *Acta Trop* 124:158-161.
- Qualls WA, Müller GC, Revay EE, Allan SA, Arheart KL, Beier, JC, Smith ML, Scott, JM, Kravchenko VD., Hausmann A, Yefremova ZA, Xue RD. 2014. Evaluation of attractive toxic sugar baits (ATSB)—barrier control of vector and nuisance mosquitoes and its effect on non-target organisms in sub-tropical environments in Florida. *Acta Trop* 131:104-110.
- Qualls WA, Müller GC, Traore SF, Traore MM, Arheart KL, Doumbia S, Schlein Y, Kravchenko VD, Xue RD, Beier JC. 2015. Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West Africa. *Malar J* 14:301.
- Revay EE, Müller GC, Qualls WA, Kline DL, Naranjo DP, Arheart KL, Kravchenko VD, Yefremova Z, Hausmann A, Beier JC, Schlein Y, Xue RD. 2014. Control of *Aedes albopictus* with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in St. Augustine, Florida. *Parasitol Res* 113:73-79.
- Revay EE, Schlein Y, Tsabari O, Kravchenko VD, Qualls W, Xue RD, Beier J, Traore SF, Doumbia S, Hausmann A, Müller GC. 2015. Formulation of attractive toxic sugar bait (ATSB) with safe EPA-exempt substance significantly diminishes the *Anopheles sergentii* population in a desert oasis. *Acta Trop* 150:29-34.
- Schlein Y, Müller GC. 2008. An approach to mosquito control: Using the dominant attraction of flowering *Tamarix jordanis* trees against *Culex pipiens*. *J Med Entomol* 45:384-390.
- Wang F, Shen Y, Dixon D, Xue RD. 2017. Control of male *Aedes albopictus* Skuse (Diptera: Culicidae) using boric acid sugar bait and its impact on female fecundity and fertility. *J Vector Ecol* 42:203-206.
- Xue RD, Barnard DR. 2003. Boric acid bait kills adult mosquitoes. *J Econ Entomol* 96:1559-1562.
- Zhu J, Zeng X, YanMa, Liu T, Qian K, Han Y, Xue S, Tucker B, Schultz G, Coats J, Rowley W. 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J Am Mosq Control Assoc* 22:515-522.
- Zhu J, Zeng X, O'Neal M, Schultz G, Tucker B, Coats J, Bartholomay L, Xue RD. 2008. Mosquito larvicidal activity of botanical-based mosquito repellents. *J Am Mosq Control Assoc* 24:161-168.

# FIELD ASSESSMENT OF AUTODISSEMINATION OF PYRIPROXYFEN BY CONTAINER-INHABITING *Aedes* MOSQUITOES IN FLORIDA

BARRY W. ALTO\*, SARA ORTIZ, KEENAN WIGGINS, CYNTHIA C. LORD,  
AND NATHAN D. BURKETT-CADENA

University of Florida, IFAS, Florida Medical Entomology Laboratory,  
200 9th St. SE, Vero Beach, FL 32962

Subject Editor: Rui-De Xue

## ABSTRACT

Domestic mosquito control for container-inhabiting *Aedes* vectors of Zika, chikungunya, yellow fever, and dengue viruses is challenging, and novel methods are needed. Autodissemination strategies are one such method. In this control method, females are attracted to stations treated with an insect growth regulator (IGR), become treated, and subsequently deposit the IGR in natural oviposition sites, preventing pupal emergence. We developed and tested treatment stations in semi-field conditions based on number of captured mosquitoes in the treatment stations. The modified treatment station attracted gravid females with oak leaf infusion and mosquito passage through exit chutes granted access to sucrose and topical contamination of IGR (pyriproxyfen) for 25% of released mosquitoes. Although a majority of released mosquitoes were uncaptured (75%), sufficient amounts of pyriproxyfen contaminated female mosquitoes to result in 75% inhibition of adult emergence in larval bioassays. These stations were then used in a field experiment to test the efficacy using sentinel cups with mosquito larvae. Three treatments which included varied numbers of autodissemination stations (control, low density, and high density) were compared. Both low and high density of stations provided high inhibition of adult emergence from sentinel cups relative to controls. We did not observe differences in mosquito emergence inhibition whether sites contained low or high densities of stations (i.e., similar rates of mosquito inhibition). Two additional field trials showed that topically contaminated mosquitoes were traveling further than expected and causing mortality in sentinel cups at least as far as 80 meters from the autodissemination stations. The development and implementation of autodissemination of IGRs is an additional tool for use in integrated mosquito management.

Key Words: Domestic mosquito vectors, emerging mosquito-borne pathogens, vector control

## INTRODUCTION

The global emergence of mosquito-borne pathogens such as Zika, dengue (DENV) and chikungunya (CHIKV) viruses in recent years have resulted in a large number of imported cases and local transmission of these three arboviruses in Florida, indicating increased risk for this region of the U.S. (Monaghan et al. 2016). These viruses are transmitted by invasive domestic mosquito species *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) which are widely dispersed throughout most of Florida (Lounibos et al. 2016). These two species have similar biological characteristics, including inhabiting containers during the immature stages and females visit multiple oviposition sites while laying eggs (skip oviposition) (Colton et al.

2003; Reiter 2007; Davis et al. 2015). Both species exhibit gonotrophic discordance whereby multiple blood feedings may occur during a single gonotrophic cycle, a behavior which enhances infection and transmission of arboviruses (Scott et al. 1993a, 1993b; Fernández and Forattini 2003). Small containers and hidden water-holding structures are notoriously difficult to find and treat using common control methods (Russell et al. 2002; Gonzalez et al. 1995; Montgomery & Ritchie 2002; Barrera et al. 2008). However, mosquito control is the principal method to reduce human-mosquito-virus contact and reduce disease transmission, so developing improved methods to find and treat these containers is critical.

Autodissemination of insect growth regulators (IGRs) is a novel approach to deliv-

ering insecticides to mosquito larval habitats through use of an autodissemination station (ADS) containing baits and insecticides such as IGRs that disrupt development of mosquitoes during the immature stages. The use of IGRs that act on the late immature stages (pupae) takes advantage of other sources of mosquito mortality in nature that limit population sizes, including density-dependent mortality (Juliano 2007). For example, larval competition and nutrient limitation still occur and cause mortality prior to the mortality caused by the IGR at the pupal stage, resulting in higher overall immature mortality. Female mosquitoes are attracted to the stations when ready to lay eggs, become contaminated with the IGR and subsequently deposit it in other oviposition sites visited later (Kartzinel et al. 2016). *Aedes aegypti* and *Ae. albopictus* females exhibit skip-oviposition, increasing dissemination of the IGR (Colton et al. 2003; Reiter 2007; Davis et al. 2017, 2015). Pyriproxyfen has been used in several autodissemination studies because it is highly effective at low concentrations (Chism and Apperson 2003; Devine et al. 2009; Gaugler et al. 2012; Kartzinel et al. 2016; Unlu et al. 2017; Suman et al. 2018; Lwetoijera et al. 2019) and is not repellent to adult mosquitoes (Sihuincha et al. 2005). Previous studies have investigated different options for the medium used to carry the IGR, allow it to be picked up by females, and the level of active ingredient needed for acceptable levels of control (Gaugler et al. 2012; Wang et al. 2014; Kartzinel et al. 2016). However, many aspects of pyriproxyfen as a control method and autodissemination approaches warrant further research and development, in order to optimize station geometry, attractants, and IGR delivery to produce designs that treat large numbers of females and achieve high levels of control (Maoz et al. 2017). Important questions remain on the density of stations needed to achieve satisfactory control and distance over which autodissemination is effective (Suman et al. 2018). Here we describe studies aimed at improving and quantifying efficacy of a prototype autodissemination station for deployment

of IGRs and assessment of the autodissemination stations in the field in Florida.

## MATERIALS AND METHODS

### *Autodissemination station optimization.*

The gravid *Aedes* (GAT) trap (Model 2797 BioQuip, Rancho Dominguez CA) was used as the overall structure of the station and was modified for each iteration of the autodissemination station development process. The capture chamber of the GAT trap (normally translucent) was painted black and a clear plastic funnel was placed at point of entry to reduce exit of females through the station entrance, a modification that was expected to increase the number of females that would exit through marking points. All stations utilized three exit chutes, consisting of vertically oriented tubes (Snap-Seal Disposable Plastic Sample Containers 02-540-15, Fisher Scientific) that serve as the location for contaminating females with pyriproxyfen powder (Fig. 1).

A series of semi-field experiments, described below, were conducted to evaluate various station parameters and select more effective characteristics with the aim of designing an attractive station that "contaminated" a maximum number of females (i.e., individuals being tainted with pyriproxyfen). Variables examined included entry and exit modifications of the station, presence and absence of sugar source, types of lures, and level of contamination with pyriproxyfen. Gravid females (3 days post bloodmeal) of *Ae. aegypti* or *Ae. albopictus* (range 128-197) were released into a screened outdoor cage (2 x 2 x 2m) with 2-3 prototype stations. Mosquitoes used in the studies were from laboratory lines established from field collections in Florida. Stations were checked daily for four consecutive days for trapped mosquitoes and to ensure the stations were functioning. In experiments 1-3, the number of females trapped was recorded daily, and the overall proportion of trapped females that were released was used to determine the relative effectiveness of modifications to the previous design. In experiment 4, females were collected daily and used in larval bioassays

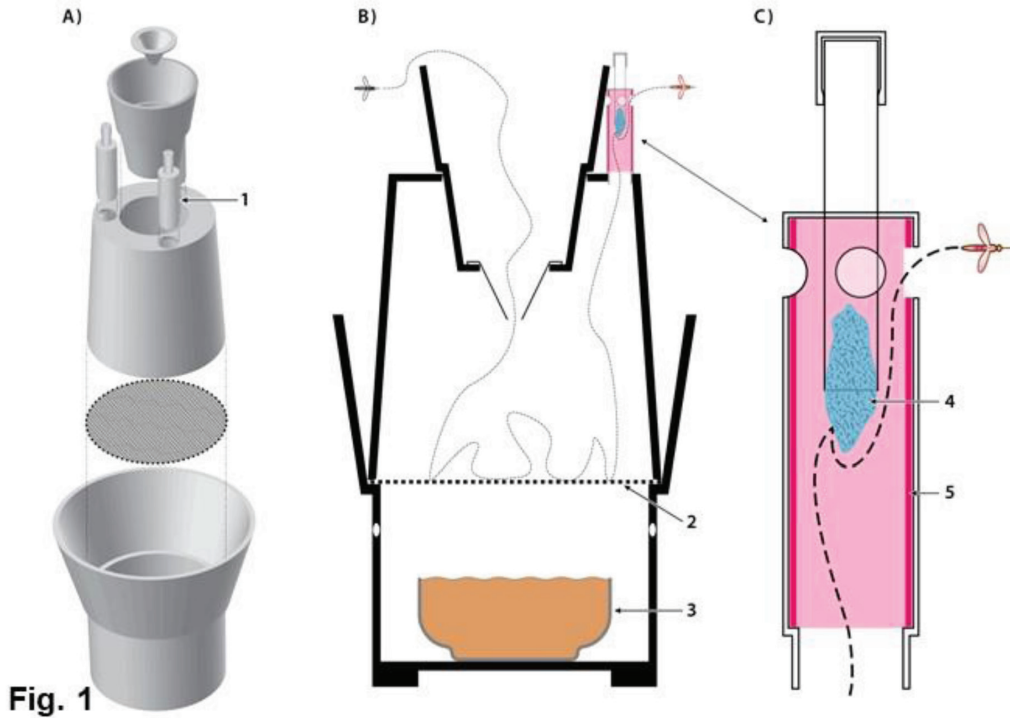


Figure 1. Autodissemination station components (A) with plastic exit chutes (1) containing pyriproxyfen. Mosquitoes enter the autodissemination station (B) and a screen (2) prevents them from access to oak leaf infusion attractant (3) and leave through the exit chute (C) which contains sucrose bait (4) and pyriproxyfen (5). Dashed lines denote the mosquito flight route.

to assess contamination with pyriproxyfen. For experiments 1-3, mesh bags were placed over exit chutes so that mosquitoes exiting through were captured for data recording (Fig. 1). Experiments were conducted over a period of four months (March-June 2016). We avoided contamination events by application of pyriproxyfen to exit chutes in a separate location compared to where semi-natural and field trials were performed. All autodissemination station parts were handled with clean disposable gloves and pyriproxyfen treated items discarded after a single use.

Experiment 1 tested the effectiveness of stations using an oviposition lure (oak leaf water infusion) versus host-seeking lure (chemical lure described by Kartzinel et al. 2016). *Ae. aegypti* and *Ae. albopictus* exhibit gonotrophic discordance and so may feed more than once during a gonotrophic cycle, responding to host attractants while gravid. Three GAT traps (modified as above, with/

out exit nets) were deployed in two separate, consecutive trials. Each trial refers to a semi-field study. In trial 1 the oviposition lure consisted of 500 mL of well water mixed with 1000 mL oak leaf infusion (O'Meara et al. 1989). Oak leaf infusion, produced using published protocols (Alto et al. 2008), is routinely used to attract gravid *Aedes* mosquitoes for oviposition (Ponnusamy et al. 2010). Oak leaves are abundant and widespread in Florida container systems occupied by these *Aedes* mosquitoes (Reiskind et al. 2009). In trial 2 the host-seeking lure (chemical lure) consisted of a mixture of 1.0 mL hexanoic acid, 13.0 mL lactic acid, 26.0 mL tap water, and 2.3 g sodium polyacrylate (gel absorbent) and a separate container with 8 g ammonium bicarbonate (Kartzinel et al. 2016). Totals of 128 *Ae. albopictus* or *Ae. aegypti* females were released into the outdoor cage for each trial.

Experiment 2 examined the effects of infusion age and of adding a sugar source

to the exit chute to draw females to the site of pyriproxyfen marking. Three GAT traps were deployed, each with three exit chutes (without pyriproxyfen) and associated "sugar wicks" in 2 trials. Each sugar wick consisted of a 5 mL plastic test tube with a cotton ball and 3 mL of 10% sucrose solution. The attractant was 1 liter of oak leaf infusion water (Trial 1, one week old and Trial 2, >2 weeks old) per station (O'Meara et al. 1989). Totals of 179 (Trial 2, >2 weeks old infusion) and 169 (Trial 1, 1-week-old infusion) *Ae. albopictus* females were released into the outdoor cages.

Experiment 3 tested the effect of adding pyriproxyfen to exit chutes (with sugar wicks) for trapping mosquitoes to assess if the presence of pyriproxyfen decreased attraction to the station. Aerosol cooking oil (PAM® Original) was applied *ad libitum* to the inner surface of each exit chute to improve adhesion of pyriproxyfen (Esteem® WP, Valent Biosciences) to mosquito cuticle. Two replicates were conducted. The first replicate employed three stations with 153 *Ae. albopictus* females. The second replicate employed two stations with 197 *Ae. albopictus* females. The attractant was 1 liter of oak leaf infusion water (one week old) per station (O'Meara et al. 1989). The goal of this experiment was simply to establish that mosquitoes would enter stations and pass through the exit chutes in the presence of pyriproxyfen, so no other treatments or controls were necessary. The subsequent experiment investigated application methods.

Experiment 4 tested the effect of two pyriproxyfen application methods for transfer of IGR to females, larval oviposition sites, and inhibition of adult emergence. Two GAT traps were deployed, each with 3 exit chutes, associated "sugar wicks" and pyriproxyfen. One station utilized exit chutes with the inner surface coated with aerosol cooking oil prior to pyriproxyfen (as in Experiment 3) while the other station utilized only pyriproxyfen dust *ad libitum* applied to the inner surface of the exit chutes. Untreated traps were not used as the goal was to compare treatment methods only. A total of 50 *Ae. albopictus* females were placed di-

rectly into the station trap chamber and the entrance closed with cotton, so that females exiting the station would pass through the exit chutes and into collection bags. These females were freeze-killed then used in larval mortality bioassays using methods described in Kartzinel et al. (2016). Briefly, females were placed in WhirlPak bags containing 100 mL water, 10 2nd-instars of *Ae. albopictus* and larval food (10 mL of food suspension consisting of equal parts yeast and lactalbumin). To avoid any potential for contamination, females from the same colony (never exposed to traps or the experimental cage) were freeze-killed and used as controls for the larval mortality bioassay. Bags were monitored for pupation, pupal death, and adult emergence.

For experiments 1-3, treatment effects (variables tested) on capture rates of mosquitoes in the autodissemination stations were analyzed using maximum likelihood categorical analyses of contingency tables (PROC CATMOD, SAS 2002) based on the number of mosquitoes recovered from the exit chutes. Some comparisons were within experiments while some were between experiments. Analysis of variance was used in experiment 4 to compare the two methods for pyriproxyfen application as measured by larval mortality bioassays, relative to controls (PROC GLM, SAS 2002). Tests of assumptions of normally distributed residuals and homogeneous variances were not significant. Post hoc tests used pairwise comparisons of treatment groups (Ryan-Einot-Gabriel-Welsch multiple stepdown procedure, SAS 2002).

#### *Field assessment of autodissemination station.*

Field experiments were performed at White City Cemetery, White City, Florida. The cemetery was chosen because it has been used in the past for collections of *Ae. albopictus* and *Ae. aegypti* and because it could be divided into areas with approximately similar environmental conditions (land cover and amount of shade). The experimental design designated three sections (approximately 60 x 100 m, with at least 20 m between sections) in the cemetery. The

maximum distance (100m) was consistent with the distance flown by most *Ae. aegypti* females, as determined by a comprehensive review of 21 mark-release-recapture studies of this vector (Harrington et al. 2005). The densities of ADS in each area were control (no stations), high density (18 stations) and low density (9 stations). The distance between treatment and control plots was approximately 80-150 meters. Stations were placed a 15 x 50 m grid, with stations at each site for high and alternating in the long dimension for Low (Fig. 2). Ten sentinel larva cups (plastic cemetery vases, Kelco) were interspersed throughout the ADSs in the high and low treatments, and in the same relative locations in the control site. Sentinel cups were placed in the field containing 10 *Ae. albopictus* first instar larvae from a lab colony, larval food, and 250 ml water. The field experiment was initiated 2 August 2016 with

placement of the ADS and sentinel cups. Exit chutes were changed and pupae removed from sentinel cups 3 times/week. To minimize potential contamination, the control (no stations) sentinel cups were handled first when visiting the site. In treatment areas, one person collected sentinel cups while another changed exit chutes. This reduced the potential for contamination of sentinel cups by personnel during experimental monitoring. Stations were visually checked for any evidence of disturbance or potential contamination by dislodged treated exit chutes; none was observed during the experiment. Pupae from sentinel cups were held in the lab until eclosion and scored for successful adult emergence or mortality. The primary outcome variable was the proportion of pupae that died (as opposed to successfully emerging as adults) from each cup on each collection day. Adult mosquitoes in the field



Figure 2. Aerial view of the cemetery with the experimental setup for treatment stations (high density, pink symbols; low density, blue symbols) and sentinel cups (yellow symbols) for the field experiment. Logistic constraints in the field situation required decreasing the long axis separation of the traps to approximately 6 meters. An array of sentinel cups (pink symbols with black dots) perpendicular to each other was used to evaluate the effect of distance of autodissemination station on mosquito mortality.



laid eggs in the sentinel cups, enabling us to characterize mortality of natural mosquito populations in addition to those larvae originally placed in the cups. We performed two replicate trials of the field experiment in August and September 2016. An analysis of variance was used to test for a treatment effect of density of ADS, time (August and September runs of the field experiment) and density by time interaction on mosquito mortality using MatLab software.

We used a field experiment to investigate the maximum distance contaminated female mosquitoes are capable of transferring pyriproxyfen to container habitats and induce mosquito mortality. We set out an array of sentinel cups with sentinel *Ae. albopictus* larvae as described in the field assessment of autodissemination experiment. Ten sentinel cup "tripods" were split evenly between two perpendicular lines extending from one edge of the high area towards the control area and away from the experimental area. The tripods consisted of 3 sentinel cups, one closed by covering with a nitrile glove and 2 open. Immatures from the open cups for one tripod were combined when sampled. This allowed comparison of mortality when potentially pyriproxyfen-contaminated females did not (covered) or did (open) have access (Fig. 4). In short, cups with larvae were arranged along an array so contaminated adult mosquitoes could access them (open cups) or were prevented from access (covered cups). All tripods were placed in shade, limiting the regularity of placement, resulting in sentinel tripods at slightly different distances from the nearest ADS (Line 1: 9.83, 28.17, 43.36, 63.83 and 80.89m; Line 2: 9.88, 25.56, 36.66, 44.6 and 52.16m, Fig. 1). Tripods were in the field for 1 week, then pupal mortality assessed in the lab. Separate regression analyses were performed on proportion of pupae dead in covered (with nitrile glove) and open sentinel cups against distance from the nearest autodissemination station. Lines were combined for analysis. If regressions were significant, slopes were compared to assess differences between open and closed cups over distance. This was intended as a pre-

liminary test of distance effects; however, a hurricane threat required dismantling the experiment and prevented further testing. Also, a separate two-tailed t-test compared mosquito mortality in open versus covered sentinel cups.

## RESULTS

### *Autodissemination station optimization.*

Experiment 1 tested the effectiveness of stations using an oviposition lure versus host-seeking lure. There were no significant effects of type of lure (host-seeking versus oviposition lure) on captures in the autodissemination stations ( $\chi^2 = 2.24$ ;  $df = 1$ ;  $P = 0.1343$ ). The autodissemination stations captured between 9.4-15.6% of released mosquitoes (Table 1).

Experiment 2 examined the effects of infusion age and of adding a sugar source to the exit chute to attract mosquitoes to the site of pyriproxyfen marking. There were no significant effects of infusion age on capture rates in the autodissemination stations ( $\chi^2 = 0.18$ ;  $df = 1$ ;  $P = 0.6702$ ). A total of 22.3-24.8% of released mosquitoes were captured in the exit chutes (Table 1). Although more mosquitoes were recovered in the exit chutes in experiment 2 than experiment 1, this result was not significant ( $\chi^2 = 0.68$ ;  $df = 1$ ;  $P = 0.4095$ , Table 1).

Experiment 3 tested the effect of adding pyriproxyfen to exit chutes for trapping mosquitoes to assess whether pyriproxyfen affects attraction to the station. There were no significant effects of addition of pyriproxyfen (pyriproxyfen or control) to exit chutes on captures ( $\chi^2 = 0.05$ ;  $df = 1$ ;  $P = 0.8241$ ). A total of 21.3-28.8% of released mosquitoes were captured in the exit chutes in experiment 3 (Table 1).

Experiment 4 tested the effect of two pyriproxyfen application methods for transfer of IGR to females, larval oviposition sites and inducing larval mortality. The test for capture rates (females exiting the trap into collection bags) showed that significantly more females were recaptured when exit chutes contained oil plus IGR than IGR

Table 1. Summary of experiments optimizing autodissemination station attractants and application methods in outdoor screen cage (2 x 2 x 2m) using gravid *Ae. albopictus* and *Ae. aegypti*. Females either entered traps freely (lure, infusion age, sugar wick and IGR repellency) or were placed in traps and collected in exit chutes (application and mortality). Percentages are combined across multiple samples.

Variable	Treatment	Species	N released	Metric	%	P
Lure	Oviposition	<i>Ae. albopictus</i>	128	Recaptures	15.6	0.1343
	Host	<i>Ae. aegypti</i>	128		9.4	
Infusion age	Old (> 2 week)	<i>Ae. albopictus</i>	179	Recaptures	22.3	0.6702
	Fresh (1 week)	<i>Ae. albopictus</i>	169		24.8	
Sugar wick	Presence	<i>Ae. albopictus</i>	169	Recaptures	24.8	0.4095
	Absence	<i>Ae. albopictus</i>	128		15.6	
IGR repellency	Presence	<i>Ae. albopictus</i>	153	Recaptures	28.8	0.8241
	Absence	<i>Ae. albopictus</i>	197		21.3	
Application method	Oil + IGR	<i>Ae. albopictus</i>	50	Recaptures	60	0.017
	Only IGR	<i>Ae. albopictus</i>	50		36	
Control	IGR	<i>Ae. albopictus</i>	50	Mortality	24	0.0174
	No IGR	<i>Ae. albopictus</i>	50		70	

only ( $\chi^2 = 5.65$ ;  $df = 1$ ;  $P = 0.0174$ , Table 1). There were significant differences observed in the immature stage mortality in bioassays between pyriproxyfen treatment and control groups ( $F_{2,39} = 5.2$ ,  $P = 0.009$ ). A significantly lower percentage mosquitoes emerged to adulthood from the larval bioassays where females passed through exit chutes treated with aerosol cooking oil and pyriproxyfen (LS mean  $\pm$  SE,  $24.0 \pm 5.8$ ) and pyriproxyfen dust (LS mean  $\pm$  SE,  $27.7 \pm 6.1$ ) than untreated controls (LS mean  $\pm$  SE,  $70.0 \pm 13.1$ ). The addition of oil did not increase pyriproxyfen loading on mosquitoes because the two application methods did not significantly differ in immature mortality measured in bioassays (Table 1).

#### Field assessment of autodissemination station.

Two replicate trials of the field experiment were conducted during August and September 2016. Pupae were removed from sentinel cups 3 times/week. Figure 3 shows the proportion of mosquito pupae dead from the sentinel cups during the field trials. Results of ANOVA showed significant effects of density treatment (number of autodissemination stations), time (August or September), and density by time interaction (Table 2) on the proportion of pupae dying. The proportion of pupae dying was significantly different be-

tween the density treatment groups, indicating an effect of the treatment stations. However, the two treatment densities were qualitatively similar, suggesting that the higher density of treatment stations did not improve control. In other words, both low and high numbers of autodissemination stations provide similar mosquito mortality in the sentinel cups. The interaction effect showed that these density treatment effects were observed in August but not in September (Fig. 3).

To determine the distance by which the autodissemination of IGR was effective, we set out an array of sentinel cups with *Ae. albopictus* larvae up to 80 meters from the treatment site containing autodissemination stations. Separate regressions were performed on proportion of pupae dead in covered and open sentinel cups against

Table 2. Analysis of variance testing for effects of density treatment of autodissemination stations, time (August and September runs of field experiment), and density by time interaction on pre-adult mortality of sentinel *Ae. albopictus*.

Source	df	F-value	P
Density treatment	2	4.93	0.0109
Time	1	62.73	<0.0001
Density x Time	2	3.18	0.0498
Error	52		
Total	57		

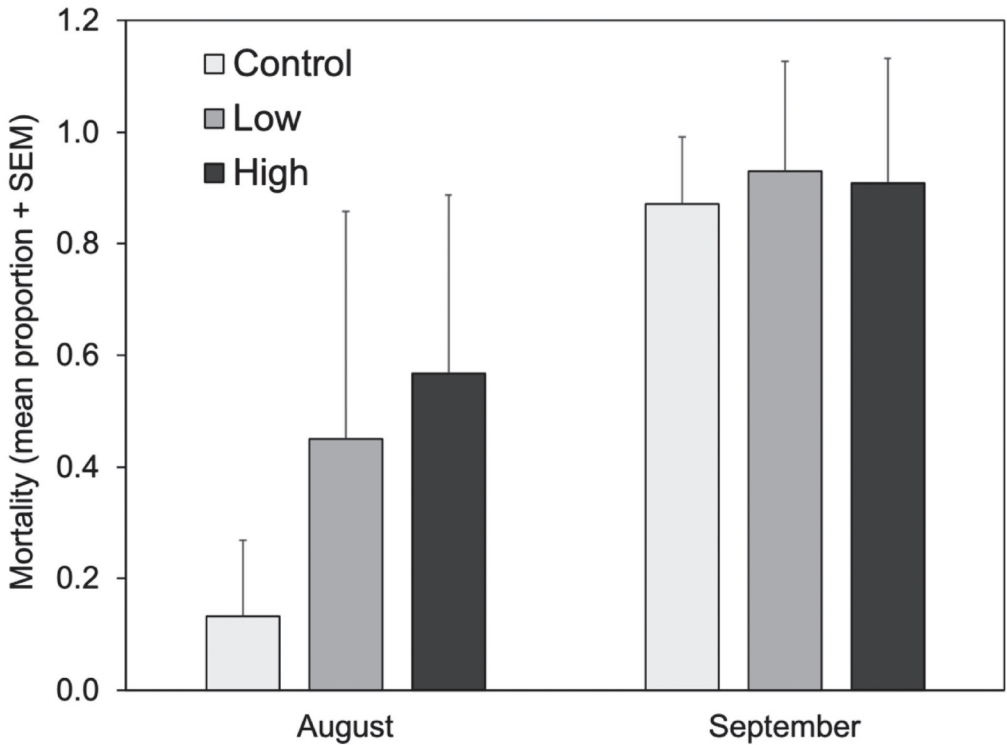


Figure 3. Effect of autodissemination station density on immature stage mortality of mosquitoes. Proportion dead pupae in sentinel cups from the field experiment in control (no stations) and treatment sites (low or high density) of autodissemination stations treated with pyriproxyfen. Two field trials were performed in August and September 2016.

distance. Neither regression was significant, indicating distance from the site with autodissemination stations did not affect pupal mortality in covered and open sentinel cups (Table 3). A separate test showed that mortality was higher in open sentinel cups than closed sentinel cups (two-tailed t-test,  $t_{18} = 2.2704$ ,  $P = 0.0357$ , Fig. 4). Increased mortality in open cups was observed up to 80m from the treatment sites.

### DISCUSSION

Here we report on the results of studies used to optimize an autodissemination station under semi-field conditions and two field trials of deployment to measure inhibition of adult emergence from sentinel larval cups. The best performing autodissemination station attracted gravid females with oak leaf infusion, although not significantly dif-

Table 3. Regression analyses on proportion of pupae dead in open and covered sentinel cups with *Ae. albopictus* against distance from a site with autodissemination stations.

Sentinel cup	Source	Estimate	Standard Error	t-statistic	P
Open	Intercept	0.4639	0.2722	1.7041	0.1268
	Distance	0.0014	0.0061	0.2236	0.8287
	Error df	8			
	Adjusted $r^2$	-0.118			
Covered	Intercept	0.1883	0.2198	0.8570	0.4164
	Distance	-0.0007	0.0049	-0.1503	0.8842
	Error df	8			
	Adjusted $r^2$	-0.122			

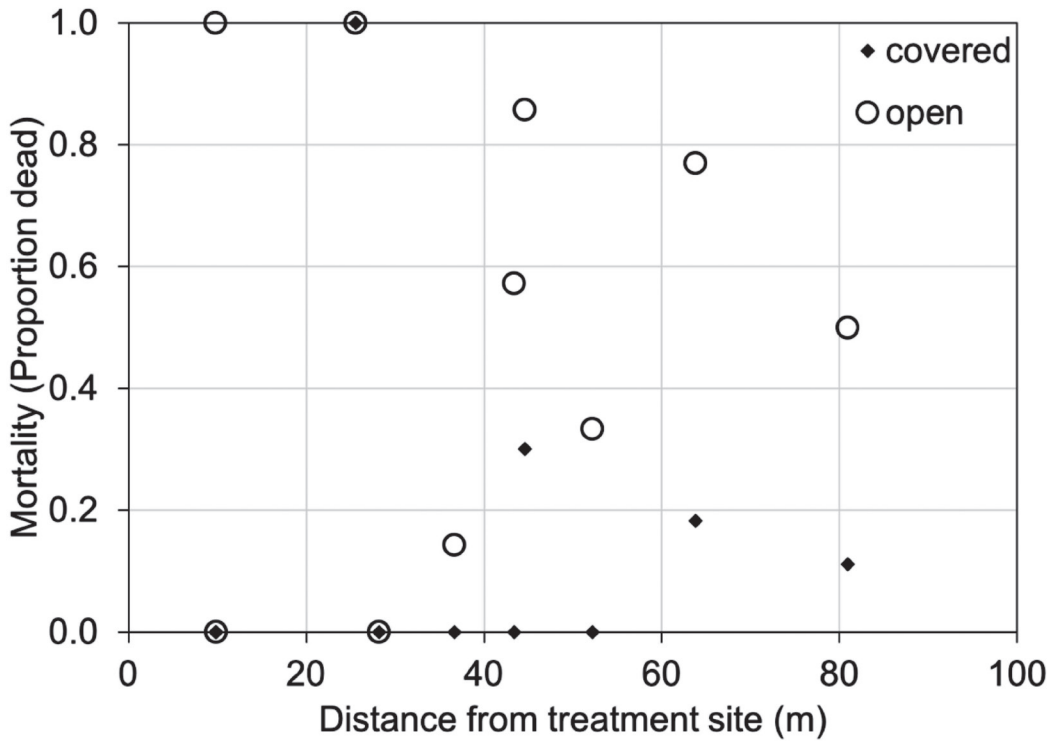


Figure 4. Relationship between distance and immature stage mosquito mortality in field assessment of autodissemination in Florida. Diamonds and circles represent covered (control) and open sentinel ovicups, respectively, used to monitor effect of pyriproxyfen on larval development. There was significantly higher mortality in open than covered sentinel ovicups (two-tailed t-test,  $t_{18} = 2.2704$ ,  $p = 0.0357$ ).

ferent from host-seeking lures. Mosquitoes enter the station through a funnel and are prevented access to the leaf infusion due to a screen barrier. Mosquitoes become topically contaminated with IGR when they leave the station through exit chutes that contain the IGR along with sucrose and oil. The narrow exit chute prevents mosquitoes from exiting by flight, and so mosquitoes are forced to walk through the exit chute which promotes tarsal contact with the IGR (Wang et al. 2014). Previous studies have shown that the efficacy of IGR rapidly declines over time under field conditions (Sullivan and Goh 2008; Kartzinel et al. 2016) and so there was a need to replace the IGR in stations. To address this issue, we included replaceable exit chutes for cost-effective and rapid renewal of IGRs in the autodissemination station without the need to move the station itself. This approach avoids the need for manipulation of pyriproxyfen and the station in the field. Rather, exit chutes can be prepared in ad-

vance and in an environmentally controlled setting, avoiding issues of manipulating pyriproxyfen in wind and rain that could otherwise result in unintended effects on non-target arthropods, such as pollinators.

Results from our semi-field trials showed that *Ae. albopictus* are attracted to the autodissemination stations. Our assessment of repellency of pyriproxyfen with *Ae. albopictus* confirmed previous findings for *Ae. aegypti* indicating lack of repellency (Sihuincha et al. 2005). Approximately 25% of released mosquitoes entered and passed through the exit chutes of the stations, ensuring topical contamination of pyriproxyfen. Low recaptures overall are due to mortality and escapes. The semi-field trials also demonstrated that sufficient amounts of pyriproxyfen topically contaminate female mosquitoes to result in approximately 75% adult emergence inhibition in bioassays. This level of emergence inhibition is similar to or higher than other observations in *Ae. albopictus* (Gaugler et al.

2012; Caputo et al. 2012; Wang et al. 2014; Chandel et al. 2016; Kartzinel et al. 2016; Buckner et al. 2017; Unlu et al. 2017; Suman et al. 2018) and other mosquito species (*Ae. aegypti*, Devine et al. 2009; Buckner et al. 2017; *Anopheles arabiensis*, Lwetoijera et al. 2014).

We anticipated that the oil formulation would enhance topical contamination of mosquitoes and facilitate rapid release into the larval habitat upon contact (Chandel et al. 2016). However, the addition of oil did not appear to increase pyriproxyfen loading because the two application methods did not differ in immature mortality measured in bioassays (Table 1). We did not assess whether the retention time of pyriproxyfen differed between the two application methods, and so it is unclear whether the addition of oil may lengthen the time pyriproxyfen remains on adult mosquitoes. The addition of oil could minimize loss of pyriproxyfen on individuals attributable to grooming behaviors (Goldman et al. 1972; Golenda and For-gash 1986; Walker and Archer 1988; Jacquet et al. 2012).

The sentinel cups containing *Ae. albopictus* immature stages used small volumes of water (250 mL) so it is unclear how effective the approach of autodissemination of IGRs would be in larger volume containers. Topical contamination of adult mosquitoes with IGRs and subsequent transfer to large volume larval rearing sites is likely to result in substantial dilution of IGRs, and so control may be limited. However, *Ae. albopictus* seems to prefer small to medium sized containers (Hawley 1988; Carieri et al. 2003) and cemetery vases are common habitats (O'Meara et al. 1995). Achieving a lethal dose may not be an issue that compromises control of this invasive species.

A distinct advantage of the autodissemination control approach is that it exploits the oviposition behavior of gravid females in nature to locate and treat larval habitats with IGRs. Additionally, the ovicidal activity of pyriproxyfen limits the potential for topically contaminated mosquitoes to further contribute to mosquito populations (Suman et al. 2013, 2015), although induction depends

on stage of ovarian development (Suman et al. 2015). For *Ae. albopictus*, oviposition in cryptic larval habitats may be favored over open containers, suggesting the former may make a substantial contribution to adult recruitment (Chandel et al. 2016). In many instances, conventional backpack and truck mounted application of larvicides are ineffective at penetrating cryptic larval habitats (Farajollahi et al. 2013; Achee et al. 2015), thus emphasizing the potential benefit of the autodissemination approach to target these mosquito habitats. We used open cups to assess efficacy of autodissemination of pyriproxyfen, and so additional studies are needed to evaluate immature stage mortality in cryptic larval habitats. A study on the efficacy of pyriproxyfen autodissemination assessed in residential areas showed similar or higher pupal mortality of *Ae. albopictus* in cryptic than open cup larval habitats (Chandel et al. 2016).

The efficacy of the autodissemination approach relies on several factors including the number of immature mosquitoes exposed to the IGR. This factor largely depends on oviposition behavior. Pheromone-like substances of larval and pupal origin have been shown in some instances to stimulate egg laying in containers inhabited by *Aedes* species (*Ae. atropalpus*, Kalpage and Brust 1973; Maire 1985; *Ae.s togoi*, Trimble and Wellington 1980; *Ae. aegypti*, Wong et al. 2011). So, the exploitation of mosquitoes for autodissemination benefits from mosquito behaviors that target container habitats suitable for development of the immature stages of mosquitoes (Wong et al. 2011). Further, skip oviposition, where females deposit eggs in multiple containers, allows for the possibility of multiple larval habitats to become contaminated with IGRs from a single topically contaminated female mosquito (Wang et al. 2014). However, in the current study, we did not specifically determine whether lethal doses of pyriproxyfen were transferred to multiple larval sites during skip oviposition.

Our work contributes to addressing several important aspects of autodissemination development, however several limitations in our study were apparent. The low percent-

ages of recovered mosquitoes (less than 25% in most treatments) in outdoor screen cage assays, for example was perplexing. Despite exhaustive searching, the majority of remaining females could not be accounted for and were presumed to have been predated by ants or died and consumed by ants or other scavenging arthropods, however this could not be confirmed. Cages were checked daily during bioassays, which was done for convenience of regular sampling. Females were removed each time and net bags replaced. More frequent checking could have resulted in greater recapture rate. The reliance upon *Ae. albopictus* in nearly all bioassays to direct decisions regarding the development of the autodissemination station may not be warranted. While ecologically similar, differences in the biology of these mosquito species are recognized and could affect optimal station design. Full field trials would have benefitted from greater temporal spacing between major replicates to ensure no residual contamination of pyriproxyfen in the environment. In addition, greater distances between ADS and sentinel cups could permit investigating the maximum distance of pyriproxyfen dispersal by mosquitoes.

We observed differences in immature stage mortality between autodissemination station densities between the two field trials. In August, we observed enhanced inhibition of adult emergence in treatment sites with both low and high densities of autodissemination stations relative to the control site, suggesting that the higher density of treatment stations did not improve control. In September we observed much higher immature stage mortality at the field sites, including the control site. The efficacy of the autodissemination control strategy is correlated with the adult population size and so enhanced mortality in September may be attributable to greater numbers of adult mosquitoes. Surprisingly, we observed high immature stage mortality at control sites. We hypothesized that topically contaminated mosquitoes were moving further than anticipated and delivering pyriproxyfen to sentinel cups in the control site. To test this hypothesis, we performed an additional field

study measuring immature stage mortality in sentinel cups along an array at increasing distances an autodissemination site. Distance from the site with autodissemination stations did not affect pupal mortality in covered and open sentinel cups with higher mortality in open sentinel cups than closed sentinel cups. Inhibition of adult emergence was observed at the furthest site (80 m), thus providing support for our hypothesis. A recent study showed that *Ae. albopictus* transferred pyriproxyfen from stations to contaminate containers and induced high immature stage mortality over 200 m (Suman et al 2018). Taken together, these results suggest that the autodissemination approach may extend the dissemination of IGR to larval habitats far from stations and substantially limit adult population sizes, especially in sites inaccessible to mosquito control personnel.

#### ACKNOWLEDGEMENTS

Jordan Vann assisted in the field experiment and James Newman assisted in preparation of figures. We thank the White City Cemetery board of directors and office manager Andrea Lawrence for allowing the field experiment at the cemetery. This study was supported by the Florida Department of Agriculture and Consumer Services contract number 022398.

#### REFERENCES CITED

- Achee NL, Gould F, Perkins TA, Reiner Jr RC, Morrison AC, et al. 2015. A critical assessment of vector control for dengue prevention. *PLoS Negl. Trop. Dis.* 9(5): e0003655. doi 10.1371/journal.pntd.0003655.
- Alto BW, Reiskind MH, Lounibos, LP. 2008. Size alters susceptibility of vectors to dengue virus infection and dissemination. *Am. J. Trop. Med. Hyg.* 79(5), 688-695.
- Barrera R, Amador M, Diaz A, Smith J, Munoz-Jordan JL, et al. 2008. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Med. Vet. Entomol.* 22:62-69.
- Caputo B, Ienco A, Cianci D, Pombi M, Petrarca V, Baseggio A, Devine JG, della Torre A. 2012. The "Auto-dissemination" approach: A novel concept to fight *Aedes albopictus* in urban areas. *PLoS Negl. Trop. Dis.* 6(8):e1793. doi:10.1371/journal.pntd.0001793.
- Carrieri M, Bacchi M, Bellini R, Maini S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environ. Entomol.* 32: 1313-1321.

- Chandel K, Suman DS, Wang Y, Unlu I, Williges E, Williams GM, R. Gaugler R. 2016. Targeting a hidden enemy: Pyriproxyfen autodissemination strategy for the control of the container mosquito *Aedes albopictus* in cryptic habitats. *PLoS Negl. Trop. Dis.* 10(12): e0005235. doi:10.1371/journal.pntd.0005235.
- Chism BD, Apperson CS. 2003. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Med. Vet. Entomol.* 17:211-220.
- Colton YM, Chadee DD, Severson DW. 2003. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Med. Vet. Entomol.* 17: 195-204.
- Davis TJ, Kaufman PE, Tatem AJ, Hogsette JA, Kline, DL. 2017. Development and evaluation of an attractive self-marking ovitrap to measure dispersal and determine skip oviposition in *Aedes albopictus* (Diptera: Culicidae) field populations. *J. Med. Entomol.* 53: 31-38.
- Davis TJ, Kaufman PE, Hogsette JA, Kline DL. 2015. The effects of larval habitat quality on *Aedes albopictus* skip oviposition. *J. AM. Mosq. Control Assoc.* 31: 321-328.
- Devine GJ, Perea EZ, Killeen GF, Stancil JD, Clark SJ, Morrison AC. 2009. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proc. Natl. Acad. Sci. USA.* 106:11530-11534.
- Farajollahi A, Williams GM, Condon GC, Kesavaraju B, Unlu I, et al. 2013. Assessment of a direct application of two *Bacillus thuringiensis israelensis* formulations for immediate and residual control of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 29:385-388.
- Fernández Z, Forattini OP. 2003. Survival of *Aedes albopictus* in Brazil: physiological age and reproductive history. *Revista de Saúde Pública* 37: 285-291.
- Gaugler R, Suman D, Wang Y. 2012. An autodissemination station for the transfer of an insect growth regulator to mosquito oviposition sites. *Med. Vet. Entomol.* 26:37-45.
- Goldman LJ, Callahan PS, Carlyle TC. 1972. Tibial combs and proboscis cleaning in mosquitoes. *Ann. Entomol. Soc. Amer.* 65:1299-1302.
- Golenda CF, Forgash AJ. 1986. Grooming behavior in response to fenvalerate treatment in pyrethroid-resistant house flies. *Entomol. Exp. Appl.* 40: 169-175.
- Gonzalez R, Suarez MF. 1995. Sewers: The principal *Aedes aegypti* breeding sites in Cali, Colombia. *Am. J. Trop. Med. Hyg.* 53(Suppl 2):160.
- Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, Jones JJ, Kitthawee S, Pattamaporn K, Sithiprasasna R, Edman JD. 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *Am. J. Trop. Med. Hyg.* 72:209-220.
- Hawley WA. 1988. The biology of *Aedes albopictus*. *J. Am. Mos. Control Assoc.* 1: (Suppl.), 1-40.
- Jacquet M, Lebon C, Lemperiere G, Boyer S. 2012. Behavioural functions of grooming in male *Aedes albopictus* (Diptera: Culicidae), the Asian tiger mosquito. *Appl. Entomol. Zool.* 47:359-363.
- Juliano SA. 2007. Population dynamics. In: Floore TG, editor. *Biorational Control of Mosquitoes*. Supplement to the Journal of the American Mosquito Control Association: *American Mosquito Control Association Bulletin* No. 7. pp. 265-275.
- Kalpage DSP, Brust RA. 1973. Oviposition attractant produced by immature *Aedes atropalpus*. *Environ. Entomol.* 2:729-730.
- Kartzinel MA, Alto BW, Deblasio MW II, Burkett-Cadena ND. 2016. Testing of visual and chemical attractants in correlation with the development and field evaluation of an autodissemination station for the suppression of *Aedes aegypti* and *Aedes albopictus* in Florida. *J. Am. Mosq. Control Assoc.* 32:194-202.
- Lounibos LP, Bargielowski I, Carrasquilla MC, Nishimura N. 2016. Coexistence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Peninsular Florida two decades after competitive displacements. *J. Med. Entomol.* 53: 1385-1390.
- Lwetoijera D, Kiware S, Okumu F, Devine GJ, Majambere S. 2014. Autodissemination of pyriproxyfen suppresses stable populations of *Anopheles arabiensis* under semi-controlled settings. *Malaria J.* 13:166.
- Lwetoijera D, Harris C, Kiware S, Dpongus S, Devine GJ, McCall PJ, Majambere S. 2014. Effective autodissemination of pyriproxyfen to breeding sites by the exophilic malaria vector *Anopheles arabiensis* in semi-field settings in Tanzania. *Malaria J.* 13: 161.
- Maire A. 1985. Effect of axenic larvae on the oviposition site selection by *Aedes atropalpus*. *J. Am. Mosq. Control Assoc.* 1:320-323.
- Maoz D, Ward T, Müller P, Runge-Ranzinger S, Toledo J. et al. 2017. Community effectiveness of pyriproxyfen as a dengue vector control method: A systematic review. *PLoS Negl. Trop. Dis.* 11(7):e0005651.
- Monaghan AJ, Morin CW, Steinhoff DF, Wilhelm O, Hayden M, Quattrochi DA, Reiskind M, Lloyd AL, Smith K, Schmidt CA, Scalf PE, Ernst K. 2016. On the seasonal occurrence and abundance of the Zika virus vector mosquito *Aedes aegypti* in the contiguous United States. *PLoS Curr.* 16:8.
- Montgomery BL, Ritchie SA. 2002. Roof gutters: A key container for *Aedes aegypti* and *Ochlerotatus notoscriptus* (Diptera: Culicidae) in Australia. *Am. J. Trop. Med. Hyg.* 67:244-246.
- O'Meara GF, Vose FE, Carlson DB. 1989. Environmental factors influencing oviposition by *Culex* (Culex) (Diptera: Culicidae) in two types of traps. *J. Med. Entomol.* 26:528-534.
- O'Meara GF, Evans LF, Gettman AD, Cuda JP. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. *J. Med. Entomol.* 32:554-562.
- Ponnusamy L, Xu N, Wesson DW, Ayyash LA, Schall C, Apperson CS. 2010. Oviposition response of the mosquitoes *Aedes aegypti* and *Aedes albopictus* to experimental plant infusions in laboratory bioassays. *J. Chem. Ecol.* 36:709-719.
- Reiskind MH, Greene KL, Lounibos LP. 2009. Leaf species identity and combination affect performance and oviposition choice of two container mosquito species. *Ecol. Entomol.* 34:447-456.
- Reiter P. 2007. Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. *Vector-borne and Zoonotic Dis.* 7: 261-273.
- Russell BM, McBride JH, Mullner H, Kay BH. 2002. Epidemiological significance of subterranean *Ae-*

- des aegypti* breeding sites to dengue virus infection in Charters Towers 1993. *J. Med. Entomol.* 39:143-145.
- Sihuincha M, E. Zamora-Perea W, Orellana-Rios J, Stancil V, Lopez-Sifuentes, et al. 2005. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J. Med. Entomol.* 42:620-630.
- Scott TW, Clark GG, Lorenz LH, Amerasinghe PH, Reiter P, Edman JD. 1993a. Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. *J. Med. Entomol.* 30:94-99
- Scott TW, Chow E, Strickman D, Kittayapong P, Wirtz RA, Lorenz LH, Edman JD. 1993b. Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. *J. Med. Entomol.* 30:922-927.
- Sullivan JJ, Goh KS. 2008. Environmental fate and properties of pyriproxyfen. *J. Pestic. Sci* 33: 339-350.
- Suman DS, Wang Y, Faraji A, Williams GM, Williges E, Gaugler R. 2018. Seasonal field efficacy of pyriproxyfen autodissemination stations against container-inhabiting mosquito *Aedes albopictus* under different habitat conditions. *Pest Manag. Sci.* 74:885-895.
- Suman DS, Wang Y, Gaugler R. 2015. The insect growth regulator pyriproxyfen terminates egg diapause in the Asian tiger mosquito, *Aedes albopictus*. *PLoS ONE* 10(6): e0130499.doi:10.1371/journal.pone.0130499.
- Suman DS, Wang Y, Bilgrami AL, Gaugler R. 2013. Ovicidal activity of three insect growth regulators against *Aedes* and *Culex* mosquitoes. *Acta Trop.* 128:103-109.
- Trimble RM, Wellington WG. 1980. Oviposition stimulant associated with fourth-instar larvae of *Aedes togoi* (Diptera: Culicidae). *J. Med. Entomol.* 17:509-514.
- Unlu I, Suman DS, Wang Y, Klingler K, Faraji A, Gaugler A. 2017. Effectiveness of autodissemination stations containing pyriproxyfen in reducing immature *Aedes albopictus* populations. *Parasites and Vectors* 10:139.
- Walker ED, Archer WE. 1987. Sequential organization of grooming behaviors of the mosquito, *Aedes triseriatus*. *J. Insect Behav.* 1:97-109.
- Wang Y, Suman DS, Bertrand J, Dong L, Gaugler R. 2014. Dual-treatment autodissemination station with enhanced transfer of an insect growth regulator to mosquito oviposition sites. *Pest Manag. Sci.* 70:1299-1304.
- Wong J, Stoddard ST, Astete H, Morrison AC, Scott TW. 2011. Oviposition site selection by the dengue vector *Aedes aegypti* and its implications for dengue control. *PLoS Negl. Trop. Dis.* 5(4): e1015. doi:10.1371/journal.pntd.0001015.



# LABORATORY TOXICITY OF MOSQUITO ADULTICIDES TO THE ASIAN TIGER MOSQUITO, *Aedes albopictus* AND THE HONEY BEE, *Apis mellifera*

HUSSEIN SANCHEZ-ARROYO<sup>1</sup>, ROBERTO M. PEREIRA,  
YONG-XING JIANG<sup>2</sup>, DANIEL DIXON<sup>3,4</sup>, RUI-DE XUE<sup>5</sup>,  
AND PHILIP G. KOEHLER

<sup>1</sup>Colegio de Postgraduados, México

<sup>2</sup>Gainesville Mosquito Control, 405 NW 39th Ave. Gainesville, FL 32609

<sup>3</sup>Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

<sup>4</sup>Current address: USDA/CMAVE, 1600 SW 23rd Dr., Gainesville, FL 32608

Guest editor: Vindhya S. Aryaprema

## ABSTRACT

*Aedes albopictus* and *Apis mellifera* were exposed to six insecticide active ingredients and five commercial insecticide formulations by topical application and insecticide-impregnated paper strips respectively to determine the differential toxicity and the potential use of the two methods in insecticide resistance monitoring surveys. By topical application deltamethrin was the most toxic active ingredient ( $LD_{50} = 0.018 \mu\text{g/g}$ ) for *Ae. albopictus* whereas chlorpyrifos was the least toxic ( $LD_{50} = 0.499 \mu\text{g/g}$ ). For *Apis mellifera*, the most toxic active ingredients were bifenthrin ( $LD_{50} = 0.047 \mu\text{g/g}$ ) and deltamethrin ( $LD_{50} = 0.055 \mu\text{g/g}$ ) while chlorpyrifos ( $LD_{50} = 0.215 \mu\text{g/g}$ ) and permethrin ( $LD_{50} = 0.287 \mu\text{g/g}$ ) had comparatively low toxicity. When the insecticide-impregnated method was used, Mosquito Mist (a.i. chlorpyrifos) was the most toxic commercial formulation for both *Ae. albopictus* ( $LC_{50} = 0.028 \mu\text{g/cm}^2$ ) and *A. mellifera* ( $LC_{50} = 0.059 \mu\text{g/cm}^2$ ). Duet and DeltaGard showed the least toxicity ( $LC_{50} = 2.429 \mu\text{g/cm}^2$  and  $LC_{50} = 0.491 \mu\text{g/cm}^2$  respectively) for *Ae. albopictus* and DeltaGard was the least toxic to *A. mellifera* ( $LC_{50} = 18.09 \mu\text{g/cm}^2$ ).

When using the topical application method with insecticide active ingredients, more than 3 times permethrin and deltamethrin were required to obtain the same mortality rate in *A. mellifera* as in *Ae. albopictus*. However, chlorpyrifos was more toxic for *A. mellifera* than for *Ae. albopictus*. In the insecticide-impregnated paper-strip method with commercial insecticide formulations, more than 36 times of DeltaGard was required to obtain the same mortality rate in *A. mellifera* as in *Ae. albopictus*. Even though the Mosquito Mist is the most toxic commercial formulation for both insect species, *A. mellifera* were more than 2 times tolerant to this insecticide compared to *Ae. albopictus*.

The study concludes the active ingredient deltamethrin or its commercial formulation DeltaGard is the best among tested insecticides to control *Ae. albopictus* with minimal effects to *A. mellifera*.

Key Words: *Aedes albopictus*, *Apis mellifera*, insecticides, toxicity, mosquito control

## INTRODUCTION

*Aedes albopictus* Skuse, also called as the Asian tiger mosquito is a widely distributed mosquito species in tropical, subtropical and temperate climate zones. It is an important vector of several viral infections, including yellow fever, dengue, chikungunya, and Zika virus. The spraying of chemical insecticides to control the vector is one of the most important methods to prevent the transmission of those arboviral diseases. Pyrethroids have been widely

used as indoor/outdoor residual or space sprays or mosquito control because of their high effectiveness. Usage of some insecticides results in unfortunate consequences to nontarget beneficial organisms such as honeybees. Honeybees are responsible for providing more than 90% of commercial pollination services in agricultural crops in the United States (Bruckner et al. 2019). The elevated loss rates seen recently in managed honeybee colonies threaten those pollination ser

vices (Lopez-Uribe and Simone-Finstrom, 2019). Therefore, there is a global concern about the decline of honeybee populations which is attributed to a range of factors such as “Colony Collapse Disorder” (Williams et al. 2010), pathogens and pesticides (Ostiguy et al. 2019). Since the worker honeybees can forage up to 12 km around their hive and reach urban areas (Beekman and Ratnieks 2000), they can be exposed to a several different insecticides. Some studies have concluded that barrier or ground insecticide applications to control host-seeking mosquitoes may affect nontarget insects such as honeybees (Qualls et al. 2010; Drake et al. 2016). Better practices should be adhered to minimize adverse effects on non-target organisms such as honeybees while implementing mosquito control with insecticides.

Increased use of insecticides leads to the progressive development of chemical insecticide resistance among mosquitoes (Knox et al. 2014) and therefore, programs using insecticides to control mosquitoes should always include insecticide resistance monitoring and management. Standard laboratory studies utilize topical bioassays, applying insecticides to the mesothoracic pleural or dorsal body regions, or the use of insecticide-impregnated papers, where insects pick up chemical on their tarsi. Both of these methods are commonly used to determine toxicity or insecticide resistance (WHO, 2009, WHO, 2018).

The objectives of this study were to determine the differential toxicity of six active ingredients and five commercial insecticide formulations on *Ae. albopictus* and *Apis mellifera* using two bioassay methods and determine their potential use in future insecticide resistance monitoring surveys. It would help mosquito control personnel to make informed decisions on the best use of insecticides that will have minimum to no effect on honeybee populations.

## MATERIALS AND METHODS

**Insects.** *Ae. albopictus* adults were obtained from colonies maintained at the USDA-ARS, Center for Medical, Agricul-

tural, and Veterinary Entomology (CMAVE) in Gainesville, FL., USA. *A. mellifera* were obtained from an apiary managed by the Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL., USA. Mosquitoes and honeybees were provided with 10% and 50% sucrose solution *ad libitum* respectively throughout the experiments. The honeybee colonies were of mixed race, European-derived stock housed in standard Langstroth-style equipment and managed according to common practices for North Central Florida.

**Active ingredient experiments.** Six commonly used mosquito adulticidal active ingredients namely, phenothrin (94.6%), prallethrin (96%), deltamethrin (99.7%), chlorpyrifos (99.3%), permethrin (96.7%) and bifenthrin (99.1%) from Sigma-Aldrich, USA were used in the experiments. The active ingredients were serial-diluted in acetone to make 1.0, 0.1, 0.01, 0.001, 0.0001, and 0.00001% dilutions. In the second part of the bioassay intermediate dilutions were included. Each dilution was applied separately on thoraxes of *Ae. albopictus* and *A. mellifera* adult females by topical application method. Ten adult female mosquitoes (3-4 days old) were knocked down exposing to CO<sub>2</sub> for 15 s for each of the 5 replicates per treatment and treated with 0.1 µl of an insecticide preparation using a 5 µl syringe (Hamilton Co. Reno NV) and a repeating dispenser (Hamilton PB 600-1). Treated mosquitoes were transferred to 20-ml scintillation vials and covered with a mesh to prevent escape. The mosquitoes of control experiments were treated with acetone only.

Ten adult worker *A. mellifera* (5-10 days old) were knocked down with CO<sub>2</sub> for 20 s for each of 5-7 replicates per treatment and treated with 1 µl of the insecticide preparation using a 50 µl syringe (Hamilton Co. Reno NV) and a repeating dispenser (Hamilton PB 600-1). They were then transferred to 120-ml glass jars and covered with a mesh. The honeybees of control experiments were treated with acetone only.

**Commercial insecticide experiments.** Five commercial insecticides; Mosquito Mist

(chlorpyrifos 24.6%), Aqualuer (permethrin 20.6%, PBO 20.6%), DeltaGard (deltamethrin 2.0%), Duet (Prallethrin 1.0% + Phenothrin 5.0%) and Talstar (Bifenthrin 7.9%) were tested using the insecticide-impregnated paper method. Serial dilutions were prepared using different diluents depending of the pesticide formulation. Mosquito Mist and Aqualuer were diluted in acetone; DeltaGard and Talstar were diluted in distilled water; and Duet was diluted in mineral oil. The diluent for the control experiments was the same for the corresponding insecticide. Different amounts of insecticide solution were applied depending on the solvent used for each commercial formulation.

Each insecticide preparation was applied to filter paper strips (Whatman filter paper # 2). For *A. mellifera* the strips were 14 cm<sup>2</sup> (2 x 7 cm) and for *Ae. albopictus* the strips were 5 cm<sup>2</sup> (1 x 5 cm). Dosages of insecticides were calculated as such to ensure the same amount of insecticides per cm<sup>2</sup> in both sizes of the paper strips (Table 1).

Ten adult *Ae. albopictus* females (3-4 days old) were knocked down using CO<sub>2</sub> for 15 s for the replicate of each concentration and transferred to 20-ml scintillation vials with mesh covers. After 30 minutes and complete recovery from CO<sub>2</sub> anesthetizing, the insecticide-impregnated paper strip was introduced to the scintillation vial. Five replicates were carried out on separated days.

Ten worker *A. mellifera* (5-10 days old) were knocked down using CO<sub>2</sub> for 20 s for the replicate of each concentration and transferred to a 120-ml glass jars with mesh covers. After 30 minutes and complete recovery from CO<sub>2</sub> anesthetizing, the insecticide-impregnated strip was introduced to the jar.

Any bees that were not walking at the time the insecticide-treated paper strip was added to the jars were not considered for the experiment. Five to seven replicates were carried out on separated days.

At least 350 each of *Ae. albopictus* and *A. mellifera* (50 control and 300 insecticide treated) were tested for mortality against each insecticide in each experiment. Mortality was assessed 24 h post exposure to insecticides. When mortality in control experiments were above 5%, mortality data of corresponding treatment experiments were corrected using Abbott's (1925) formula before calculating LD<sub>50</sub> or LC<sub>50</sub>. LD<sub>50</sub> or LC<sub>50</sub> values were compared to determine the differential toxicity of insecticides to the two species. Data were analyzed by probit analysis and significance was assessed by the degree of overlap of 95% CI (SAS 9.4 Institute Inc., Cary, NC).

## RESULTS

**Toxicity of the active ingredients.** Deltamethrin was the most toxic (LD<sub>50</sub> = 0.018 µg/g) among the 6 tested adulticidal active ingredients when applied topically on *Ae. albopictus* followed by bifenthrin (LD<sub>50</sub> = 0.029 µg/g), permethrin (LD<sub>50</sub> = 0.076 µg/g) (Table 2). Chlorpyrifos was the least toxic active ingredient (LD<sub>50</sub> = 0.499 µg/g) for this mosquito species.

For *A. mellifera*, the most toxic insecticides were bifenthrin (LD<sub>50</sub> = 0.047 µg/g) and deltamethrin (LD<sub>50</sub> = 0.055 µg/g), with no significant differences between them, followed by phenothrin (LD<sub>50</sub> = 0.131 µg/g). The least toxic active ingredient for *A. mellifera* was prallethrin (LD<sub>50</sub> = 0.779 µg/g) which is normally added to commercial formulations only to produce the knockdown effect because of its low toxicity. Chlorpyrifos (LD<sub>50</sub> = 0.215 µg/g), permethrin (LD<sub>50</sub> = 0.287 µg/g) had low toxicity to *A. mellifera*. Both insect species had similar susceptibility to phenothrin, with LD<sub>50</sub>s of 0.186 µg/g for *Ae. albopictus* and 0.131 µg/g for *A. mellifera*.

*A. mellifera* were 3.78X, 3.06X, and 3.04X more tolerant to permethrin, deltamethrin and prallethrin respectively compared to *Ae. albopictus* (Table 2) when using topi-

Table 1. Amounts of different insecticides used to impregnated the paper strips.

Insecticide	Amounts used (µl)	
	<i>Ae. albopictus</i>	<i>A. mellifera</i>
Mosquito Mist	32	90
AquaLure	32	90
DeltaGard	50	140
Talstar	50	140
Duet	25	70



Table 3. Lethal doses (LC<sub>50</sub>) of commercial insecticides exposed to *Aedes albopictus* and *Apis mellifera* adults by insecticide-impregnated paper method.

Insecticide	<i>Aedes albopictus</i> (A)				<i>Apis mellifera</i> (B)				LC50 (A) / LC50 (B)
	LC50 (µg/cm <sup>2</sup> )	95% CI	Slope (SE)	N	LC50 (µg/cm <sup>2</sup> )	95% CI	Slope (SE)	N	
Mosquito Mist	0.028	0.025-0.032	3.030 (0.297)	300	0.059	0.036-0.113	1.78 (0.323)	300	2.11
Talstar	0.291	0.261-0.326	0.326 (0.313)	350	0.243	0.211-0.282	2.67 (0.277)	300	0.84
Aqualuer	0.423	0.35-0.599	1.440 (0.168)	300	0.773	0.618-0.962	1.71 (0.149)	350	1.83
DeltaGard	0.491	0.321-0.822	0.709 (0.086)	350	18.09	13.39-18.68	2.31 (0.257)	300	36.84
Duet	2.429	2.144-2.795	2.640 (0.242)	420	1.221	1.051-1.406	2.69 (0.281)	300	0.50

both species. One possible reason for evidenced lower toxicity of pyrethroid insecticides, when exposed to impregnated paper strips, could be the irritation they produce being kept the insects from getting in touch with the paper strips for a period longer enough to pick up the insecticide. Since, chlorpyrifos does not causes irritation the insects move freely around the insecticide-impregnated papers until they get a lethal dose.

The insecticide-impregnated paper method was originally developed to evaluate discriminating doses. In this method, the more the exposed insects move on the paper the more insecticide they pick up by their tarsi. Additionally, it has been reported that the insecticide applied to the mosquito tarsomeres of the hind leg spread out across all the tarsomeres, the tibia, and a portion of the femur of the hind leg (Aldridge et al. 2016). Both permethrin (pyrethroid) and malathion (organophosphate) contacted through appendages such as the leg has resulted in much lower mortality (Aldridge et al. 2016). Unlike in the insecticide -impregnated paper method, the topical application allows the direct absorption of applied insecticide and therefore more appropriate for the determination of toxicity of pyrethroid insecticides.

On that basis, among the tested insecticides, deltamethrin or the commercial formulation DeltaGard would be the best for controlling *Ae. albopictus* with minimal effects on *A. mellifera*. However, Mosquito Mist, the commercial formulation of chlorpyrifos and Aqualuer, the commercial formulation of permethrin, would be considered as optional insecticides for resistance management. Previous studies have reported LD<sub>50</sub> 0.59 µg/g (Greig-Smith et al. 1994) and LD<sub>50</sub> range from 0.59 to 1.14 µg/g (Hardsome and Scott 2010) of chlorpyrifos for honeybees which are very similar to those reported in the present study (LD<sub>50</sub> 0.499 µg/g). Previously reported LD<sub>50</sub> values of permethrin for honeybees are 1 µg/g (Inglesfield, 1989) and 0.15 µg/g (Danka 1986). Compromisingly, our study reports an intermediate value of LD<sub>50</sub> of 0.287 µg/g.

Considering that pyrethroids are the most common insecticides used for adult mosquito control, and the honeybees are moderately sensitive to deltamethrin and permethrin (Hardstone and Scott 2010), Al-Naggar et al. (2015) suggested that the application of these insecticides when pollinators are not foraging is an important step in avoiding unnecessary exposure of bees. Correct application timing combined with better insecticide application techniques can further increase safety of mosquito adulticidal applications on non-target insects. Aerial ultra-low volume applications using high-pressure nozzle system reduces environmental insecticides contamination and lead to decreased bee mortality (Zhong et al. 2004). Similar studies can lead to improved application techniques that can be used in the control of mosquitoes in the field with lower risk for honeybees.

Atkins et al. (1973 and 1975, cited by Danka et al. 1986) reported that the majority of referenced insecticide results are topical or contact, and the LD<sub>50</sub> obtained by topical application are relatively lower, and Felton et al. (1986) suggested that the data on the acute contact and oral toxicity of pesticides to honeybees should be expressed as LD<sub>50</sub> and should be considered as one of the elements for assessment of danger to foraging honeybees. However, our study showed that use of insecticide-impregnated papers may be better to reduce the effect on non-target species. This is critical because the honeybee genome is deficient in a number of genes encoding detoxification enzymes (Claudianos et al. 2006), therefore laboratory testing of insecticides against honey bees must guarantee exposure to the pesticides in order to avoid optimistic results.

#### ACKNOWLEDGMENTS

We thank Jamie Ellis and Branden Stanford (both from Entomology and Nematology Department, University of Florida, USA) for supplying *A. mellifera* and to the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) for supplying *Ae. albopictus*. The research reported in this

publication was supported by the Florida Department of Agriculture and Consumer Services (FDACS) under the grant fund number 025380. The content is solely the responsibility of the authors and does not necessarily represent the official views of the FDACS.

#### REFERENCES CITED

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18:265-267.
- Aldridge RL, Kafman PE, Bloomquist JR, Gezan SA, Linthicum KJ. 2016. Impact of topical application site on the efficacy of permethrin and malathion to *Culex quinquefasciatus*. *J. Am. Mosq. Control Assoc.* 32:300-307.
- Al Naggar Y, Codling G, Vogt A, Naiem E, Mona M, Sief Aa, Giesy JP. 2015. Organophosphorous insecticides in honey, pollen and bees (*Apis mellifera* L.) and their potential hazard to bee colonies in Egypt. *Ecotoxicology Environmental Safety.* 114:1-8.
- Beekman M, Ratnieks FLW. 2000. Long term foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology.* 14:490:496.
- Bruckner S, Steinhauer N, Rennich K, Aurell SD, Caron DM, Ellis JD, Fauvel AM, Kulhanek K, Nelson KC, Rangel J, et al. Honey Bee Colony Losses 2017–2018: Preliminary Results. Bee Informed Partnership. 2018. Available online: <https://beeinformed.org/results/honey-bee-colony-losses-2017-2018>. Preliminary-results/ (accessed on 13 June 2019).
- Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, Berenbaum MR, Feyereisen R., Oakeshott JG. 2006. A deficit of detoxification enzymes: Pesticide sensitivity and environmental response in the honey bee. *Insect. Mol. Biol.* 15:615-636.
- Danka RG, Rinderer TE, Hellmich RL, Collins AM. 1986. Comparative toxicities of four topically applied insecticides to Africanized and European honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 79:18-21.
- Drake LL, Gibson J. Smith M.L, Farooq M, Sallam MF, Xue, RD 2016. Evaluation of deltagard ground application against *Aedes albopictus* in a residential arena in St Augustine, Florida. *J. Am. Mosq. Control. Assoc.* 32(2):160-162.
- Felton JC, Oomen PA, Stevenson JH. 1986. Toxicity and hazard of pesticides to honey bees: Harmonization of test methods. *Bee World:* 67:114-124.
- Greig-Smith PE, Thompson HM, Hardy AR, Bew MH, Findlay E, Stevenson JH. 1994. Incidents of poisoning of honeybees (*Apis mellifera*) by agricultural pesticides in Great Britain 1981-1991. *Crop Prot.* 13 (8):567-581
- Hardstone MC, Scott JG. 2010. Is *Apis mellifera* more sensitive to insecticides than other insects?. *Pest Manag Sci:* 66:1171-1180.
- Inglesfield C. 1989. Pyrethroids and terrestrial non-target organisms. *Pestic. Sci.* 387-428.
- Knox TB, Juma EO, Ochomo EO. et al. 2014. An online tool for mapping insecticide resistance in major Anopheles vectors of human malaria parasites and review of resistance status for the Afrotropical region. *Parasites & Vectors* 7:76.

- López-Urbe MM, Simone-Finstrom M. 2019. Special issue: Honey bee research in the US: current state and solutions to beekeeping problems. *Insects* 10, 22; doi:10.3390/insects10010022.
- Ostiguy N, Drummond FA, Aronstein K, Eitzer B, Ellis JD, Spivak M, Sheppard WS. 2019. Honey exposure to pesticides: a four year nationwide study. *Insects*. 10: 13; doi:10.3390 / insects10010013.
- Qualls WA, Xue RD, Zhong H. 2010. Impact of bifenthrin on honeybees and *Culex quinquefasciatus*. *J. Am. Mosq. Control. Assoc.* 26:223-225.
- WHO. 2009. Guidelines for efficacy testing of insecticides for indoor and outdoor ground-applied space spray applications. WHO/HTM/NTD/WHOPES/2009.2. WHO. Control of Neglected Tropical Diseases. WHO Pesticide Evaluation Scheme.
- WHO. 2018. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Second Edition. World Health Organization. ISBN 978 92 4 151157 5 (NLM classification: WA 240)
- Williams GR, Tarpay DR, van Engelsdorp D, Chauzat MP, Cox-Foster DL, Delaplane KS, Neumann P, Pettis JS, Rogers REL, Shutler D. 2010. Colony collapse disorder in context. *Bioassays* 32. 845-846.
- Zhong H, Latham M, Payne S, Brock C. 2004. Minimizing the impact of the mosquito adulticide naled on honey bees, *Apis mellifera* (Hymenoptera: Apidae): Aerial ultra-low-volume application using a high-pressure nozzle system. *J. Econ. Entomol.* 97:1-7.

# EVALUATION OF PYRETHROID AND BOTANICAL BARRIER INSECTICIDES AGAINST *Aedes albopictus* IN THE LABORATORY AND FIELD

STEVEN T. SMOLEROFF, CHRISTOPHER S. BIBBS, AND RUI-DE XUE  
Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Subject Editor: Seth Britch

## ABSTRACT

Outdoor residual insecticide applications are useful for preventing or reducing mosquito populations at focal areas. Until recently, pyrethroids have been the only option for barrier sprays in mosquito control. In this study, three pyrethroid (Onslaught, Cymic CS, DeltaGard) and two botanical (Nature-Cide, Essentria IC<sup>3</sup>) outdoor residual insecticides were comparatively tested at low, mid, and high label rates against adult *Aedes albopictus* in both laboratory bioassays and field trials in St. Augustine, FL, from May-August 2017. Bioassays indicated NatureCide and Cymic CS were the most toxic across all three dilution ratios followed by DeltaGard, Onslaught, and Essentria IC<sup>3</sup>, respectively. In field trials Nature-Cide and Onslaught were the only products that reduced mosquito abundance at the low rate. However, at the mid rate NatureCide and Onslaught caused ~90% percent reduction of adult female *Ae. albopictus* in the field, the highest of all tested products. The performance of DeltaGard (79% reduction in field counts), Essentria IC<sup>3</sup> (64%), and Cymic CS (36%) in the field were not similar to the laboratory results. The universally high performance of Nature-Cide indicates that mosquito control operations should expand consideration to botanical based insecticides for field operations.

Key Words: *Aedes albopictus*, mosquito, barrier treatments, pyrethroid, essential oils, passive control

---

## INTRODUCTION

The Asian tiger mosquito *Aedes albopictus* (Skuse) is a highly invasive, peridomestic vector of arboviruses such as dengue and chikungunya (Derraik and Slaney 2015, Wilson and Chen 2015). Its adaptability and vector potential have rendered it a major public health concern while steadily increasing the global burden of vector-borne disease (Bonizzoni et al. 2013). Vector-borne diseases are responsible for more than 17% of all infectious diseases worldwide (World Health Organization 2017a). An estimated 1.38 million suspected cases of chikungunya have been recorded around the world within the last decade (World Health Organization 2017b), and during the 2016 worldwide dengue outbreak the Americas alone reported more than 2.38 million cases (World Health Organization 2017c). Targeting adult mosquito vector populations is still a key process to reduce arbovirus transmission (Manica et al. 2016).

Ground adulticide methods such as applications of a barrier treatment have com-

monly been used as part of integrated mosquito management (Brown and Xue 2011). Barrier treatments are designed to stop adult mosquitoes entering areas typically used for outdoor human activity while also reducing the need to retreat the area (Fulcher et al. 2008) and treatments have been shown to be effective for focal mosquito control in these areas (Doyle et al. 2009, Brown and Xue 2011, Conover et al. 2015). Many species of adult mosquitoes such as *Ae. aegypti* (L.), utilize foliage structures for a variety of purposes ranging from sheltered resting sites to sources of food (Xue 2008), so barrier treatments leverage resting and feeding behaviors to maximize mosquito-insecticide contact (Fulcher et al. 2008).

Public health mosquito control in the US is restricted to only two classes of mosquito adulticide active ingredient, pyrethroids and organophosphates, which limits the options available for avoiding the evolution of resistance. For example, the majority of outdoor residual insecticides contain synthetic pyrethroid active ingredients such as bifenthrin,



deltamethrin, sumithrin, or permethrin. Fortunately, recent work improving the emulsification of essential oils has enhanced development of plant-derived active ingredients, including synergy with existing active ingredients in adulticides and larvicides (Dias and Moraes 2013, Norris et al. 2015, Gross et al. 2017). Botanical “green” alternative insecticides are appealing due to their minimum risk classification, which allows more flexible reapplication procedures and more transparency about all ingredients in a product. To explore and evaluate available EPA exempt barrier insecticidal sprays in comparison with common pyrethroid products, we investigated the relative capabilities of three pyrethroids (type I and type II) and two botanical “green” alternative adulticides for control of adult *Ae. albopictus* through laboratory bioassays and field trials.

## MATERIALS AND METHODS

We obtained *Aedes albopictus* for this study from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, FL. Mosquitoes had been maintained in CMAVE insectaries at 26.6 °C, 85± 5% relative humidity (RH), 14 h light:10 h dark photoperiod, and fed on a 10% sucrose solution (Gerberg et al. 1994). Subjects used in bioassays were female, not blood-fed, and 6–8 days old.

We tested five barrier treatment formulations: Nature-Cide All Purpose Concentrate (0.5% clove and 0.5% cottonseed oil; Pacific Shore Holdings, Inc., Canoga Park, CA), Essentria IC-3 (10% rosemary, 5% geraniol, 2% peppermint oil; Envincio LLC, Schaumburg, IL), Onslaught (6.4% esfenvalerate, a type I pyrethroid; McLaughling Gormley King Company, Minneapolis, MN), DeltaGard (2% deltamethrin, a type II pyrethroid; Bayer Environmental Science, Research Triangle Park, NC), and Cyzmic CS (9.7% lambda-cyhalothrin, a type II pyrethroid; Control Solutions, Inc., Pasadena, TX). Each product was tested using label prescribed low, mid, and high application rates across separate trials.

For laboratory bioassays, we designed a cylindrical chamber using a 55 mL petri dish base covered with an inverted 266 mL (9 oz) polystyrene cup (Fig. 1). We used a hot metal probe to melt a hole through the base of the cup for aspiration and to support a sucrose solution wick, and several smaller holes around all sides of the cup for ventilation. For each of the low, mid, and high label rates, we applied 1 mL of formulation diluted in reverse osmosis (RO) water with a pipette to filter paper (Whatman No. 1; GE Healthcare Bio-Sciences, Pittsburgh, PA) 24 h in advance of bioassays. Controls consisted of RO water with no formulation. To begin the bioassay trials we placed treated filter papers into Petri dish bases and covered with the ventilated cups, with the cup then taped to the base as shown in Fig. 1. We introduced 15 adult female mosquitoes to each cup and fitted cotton balls saturated with 10% sucrose solution in the aspiration hole. We recorded total knockdown at 30 min and mortality at 24 hours. For each repetition we used 3 cups per formulation and five control



Figure 1. Bioassay chamber constructed of a Petri dish base, a pesticide-treated filter paper nested in the dish, and a ventilated polystyrene cup with sucrose solution wick, and containing 15 non-blood-fed, 5-7 d old female *Aedes albopictus* (Skuse).

cups, and conducted 3 repetitions per low, mid, and high label rates.

For field tests, we selected 10 suburban sites (5 treatment paired with 5 control) in St. Augustine, FL, similar to the one shown in Fig. 2 based on the presence of harborage suitable for *Ae. albopictus*, with a minimum of 402 m between each paired treatment and control site. Each site was an average distance of 2.2 km from a central weather station where we recorded weekly rainfall summaries (Fig. 4) to provide context for patterns of mosquito population change across all sites. We conducted 3 weeks of pre-treatment surveillance at each site using BioGents Sentinel (BGS) mosquito traps (BG-2; BioGents AG, Regensburg, Germany) baited with CO<sub>2</sub> for 24 h per week to confirm presence of *Ae. albopictus* at all treatment and control sites. We identified collections from each trap weekly and continued surveillance in this way for the duration of the study.

We used a battery powered backpack sprayer (REC 15 ABZ; Birchmeier Sprühtechnik AG, Stetten, Switzerland) to apply the barrier treatments at the 5 sites, with the machine set to 5 bar flow pressure to achieve a 1,350 mL/min flow rate. We delivered each treatment at an approximately 7-8 km/h walking pace and calibrating each formulation-rate to a 450 mL application. Each site received separate but consecutive treatments for the low, mid, and high rates, in that order, with each rate left in place with surveillance for 4 weeks. We randomly assigned the 5 formulations to the 5 treat-

ment sites, one formulation per site. Following each treatment we flushed the backpack sprayer with 3.785 L of water to prevent cross-contamination among formulations.

We analyzed laboratory bioassay data using an ANOVA and Tukey's HSD. For the field data, we used Mulla's formula (Mulla et al. 1971) to calculate the percent reduction in the relative abundance of wild mosquitoes as measured by adult surveillance:  $\%R = 100 \times [(C_1/T_1) \times (T_2/C_2)] \times 100$ ; where  $C_1$  = pre-treatment measure of mosquito abundance in the associated control site,  $C_2$  = post-treatment mosquito abundance in the control site,  $T_1$  = pre-treatment mosquito abundance in the treated site, and  $T_2$  = post-treatment mosquito abundance in the treated site. We also analyzed adult surveillance with a generalized linear model to investigate differences among treatments relative to time elapsed during the study.

## RESULTS AND DISCUSSION

Results from the laboratory bioassays are summarized in Fig. 3. We found significant performance differences among the 5 formulations for both knockdown ( $F = 11.67$ ,  $df = 4, 44$ ,  $P < 0.0001$ ) and mortality ( $F = 28.39$ ,  $df = 4, 44$ ,  $P < 0.0001$ ). Nature-Cide and Cyzmic CS caused the highest knockdown across all three dilution rates with 20-50% knockdown at the low rate, 100% knockdown at mid and high rates, and a mean mortality of  $\geq 90\%$  at all rates. DeltaGard, Onslaught, and Essentria IC<sup>3</sup> had 0% knockdown and less than 20% mortality at the low rate. DeltaGard performed better at mid and high rates than Onslaught and Essentria IC<sup>3</sup>, with the latter two formulations performing poorly overall.

Analysis of field collections indicated significantly different performance among the 5 formulations ( $\chi^2 = 10148$ ,  $df = 15$ ,  $P < 0.0001$ ). Weekly changes in relative abundance of adult *Ae. albopictus* at field sites are shown in Fig. 4. Unfortunately, we were not able to conduct field trials at the high label rate because of limitations of time. Collections of adult female *Ae. albopictus* from Nature-Cide and Onslaught treat-



Figure 2. Image of representative suburban field site selected based on the presence of suitable harborage for *Ae. albopictus* such as moderate to dense foliage, many adult resting areas, and various artificial containers for development of immature mosquitoes.

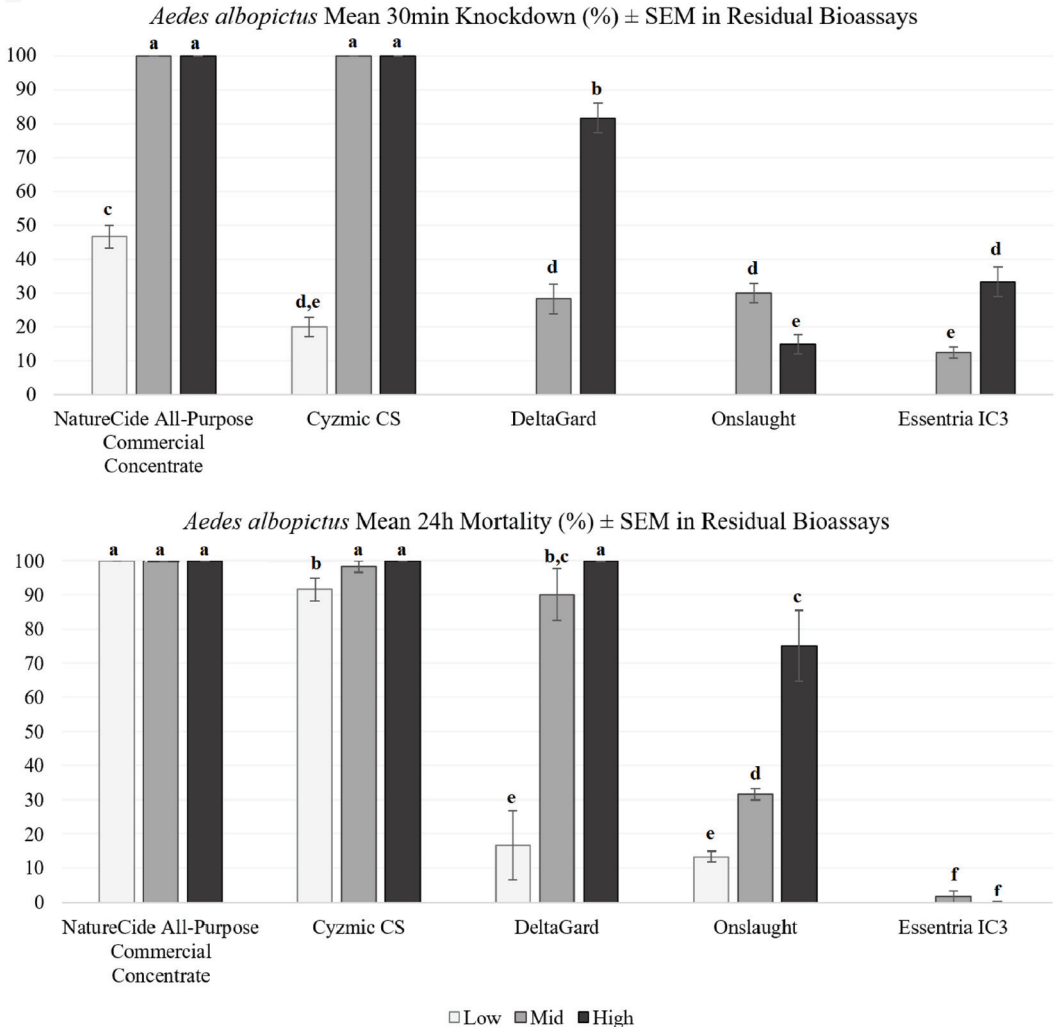


Figure 3. Average percent 24 h mortality with standard errors of the mean (ANOVA/Tukey HSD at 95% confidence,  $P < 0.001$ ) of *Aedes albopictus* (Skuse) for each of five residual spray formulations, NatureCide All-Purpose Commercial Concentrate (clove oil, cottonseed oil; 25-100 mL/L), Cyzmic CS (lambda-cyhalothrin; 1.5-3.0 mL/L), DeltaGard (deltamethrin; 2-12 mL/L), Onslaught (fenvalerate; 4-8 mL/L), and Essentria IC<sup>3</sup> (Rosemary oil, peppermint oil; 23-47 mL/L) applied at low, mid, and high label rates. Control bioassays produced 0% mortality.

ment sites showed a net reduction of 80% by Week 8 (i.e., 4 weeks post treatment with the low rate). On the other hand, after 4 weeks with the low rate the site treated with Cyzmic had no meaningful change in relative abundance, while sites treated with DeltaGard and Essentria IC<sup>3</sup> had a net increase in *Ae. albopictus* between 10% and 20%. With mid-rate applications, however, sites treated with Nature-Cide and Onslaught had 90% net reductions in mosquito collections 4 weeks post treatment, compared to DeltaGard (79% net reduction),

Essentria IC<sup>3</sup> (64%), and Cyzmic (36%). In the GLM for the week-by-week comparison the treatment used ( $\chi^2 = 6554.87$ ,  $df = 5$ ,  $P < 0.0001$ ) explained most of the variation, followed by the duration of weeks across the study ( $\chi^2 = 3593.13$ ,  $df = 10$ ,  $P < 0.0001$ ).

It was surprising to find that Nature-Cide, formulated with clove and cottonseed oil as a multi-purpose insecticide, outperformed all other products in both laboratory (Fig. 3) and field (Fig. 4) trials. In contrast, the other tested botanical product, Essentria IC<sup>3</sup>, had zero to low effects

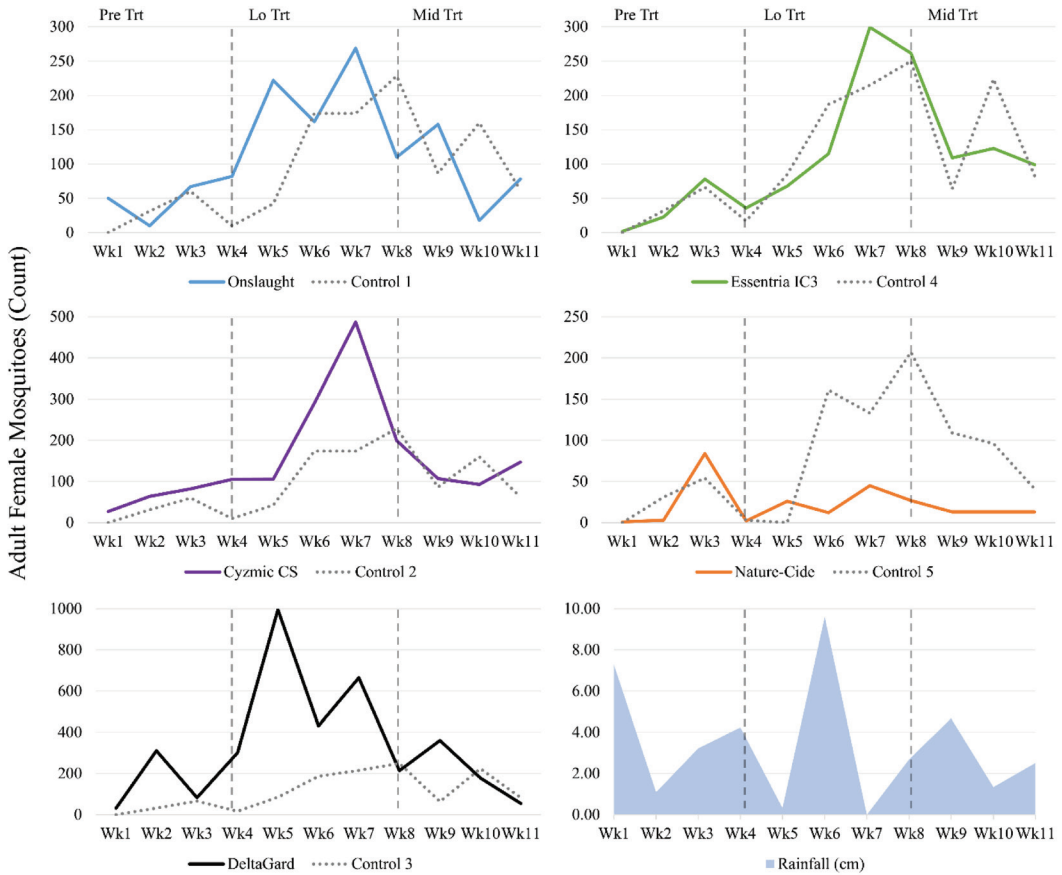


Figure 4. Field collections of adult *Ae. albopictus* from 5 sites treated with residual insecticides paired with 5 untreated control sites, with rainfall data (cm) from a centrally located weather station to provide context for patterns of mosquito population change. Each graph includes results from collections for 3 weeks prior to application of the residual treatment that confirmed presence of *Ae. albopictus* at all treatment and control sites. We initiated experimental treatments at Week 4 with the low rate which, with the exception of Nature-Cide, did not substantially reduce *Ae. albopictus* abundance. We applied mid rate treatments at Week 8 resulting in *Ae. albopictus* reduction at all treatment sites: Nature-Cide and Onslaught (~90% reduction), DeltaGard (~79%), Essentria IC3 (64%), and Cyzmic CS (~36%). We were not able to conduct field trials at the high label rate because of time limitations. Reduction was quantified using Mulla's formula (see text for details).

in laboratory bioassays yet low to moderate efficacy for reducing field populations of *Ae. albopictus* which could imply effects besides toxicity in a field environment. The rosemary, geraniol, and peppermint in Essentria IC<sup>3</sup> could be stronger as repellents than insecticides, but we did not collect outside the treatment sites to determine if mosquito populations in adjacent areas may have increased. In comparison, the very high efficacy of Cyzmic CS, DeltaGard, and Onslaught in laboratory bioassays was not mirrored in field collections. Cyzmic CS and DeltaGard, both containing type II pyrethroids, completely failed to reduce

mosquitoes when applied at the low label rate and at the mid rate performed below Onslaught, the only type I pyrethroid formulation we tested.

Pyrethroids are the most commonly used insecticides for adult mosquito control because of low environmental impact, high insecticidal potency, and good mammalian safety profiles (Amoo et al. 2008). However, the Federal, Insecticide, Fungicide, and Rodenticide Act (FIFRA) restricts the frequency that pyrethroids may be applied to the environment for adult mosquito control, spurring demand for research emphasizing green chemistry. The

Environmental Protection Agency (EPA) allows minimum risk pesticides to be exempt from FIFRA (40 C.F.R. §152.25 2015). Therefore, exempt pesticides containing for example the botanical ingredients described above can be applied more frequently than FIFRA labeled products. This intrinsically appeals to mosquito control programs when treatments need frequent reapplication, for example during significant mosquito outbreaks or when mitigating arbovirus transmission. Furthermore, exempt pesticides could provide different chemical classes for mosquito control programs, potentially reducing the risks of both resistance and environmental impact.

In the literature there are recent and accumulating examples of botanical oils used for mosquito control, with various ingredients functioning as repellents (Gross and Coats 2015), enhancers of other active ingredients (Gross et al. 2017), or acting as a synergist for toxicity (Tong and Bloomquist 2013, Gross et al. 2017). Plant-derived active ingredients for pesticides have generated enough interest to prompt the screening of 361 essential oils from 269 plant species as larvicides against *Ae. aegypti* (L.) (Dias & Moraes 2013). Phytochemicals have also become important in adulticide development due to the success of microemulsion formulations (Montefuscoli et al. 2013, Gross et al. 2017). Commercially available plant essential oils have been screened as adulticides against *Ae. aegypti* and *Anopheles gambiae* Say with favorable results (Norris et al. 2015). Despite these impressive developments centered on plant-derived compounds for public health vector control, key botanically based products suitable for mosquito control programs such as ultra-low volume (ULV) cold aerosol space sprays are not yet developed for operational use. The positive results using Nature-Cide as an outdoor residual treatment in this study demonstrate that botanically based formulations are ready to be investigated further and possibly incorporated operationally into mosquito control programs.

## ACKNOWLEDGEMENTS

We thank Jason Conrad and Univar for their assistance with selecting and sourcing the formulations chosen for this study. This is a research report only; specific mention of commercial products does not imply endorsement by the Anastasia Mosquito Control District.

## REFERENCES CITED

- Amoo AOJ, Xue RD, Qualls, WA, Quinn BP, Bernier UR. 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
- Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends Parasitol*, 29(9):1-19.
- Brown JR, Xue RD. 2011. Ground application of Aquareslin and Aquakontrol against *Anopheles quadrimaculatus*, *Aedes aegypti*, and *Aedes albopictus*. *J Am Mosq Control Assoc* 27:330-332.
- Conover D, Fulcher A, Smith ML, Farooq M, Gaines MK, Xue RD. 2015. Evaluation of three commercial backpack sprayers with Aqualuer® 20-20 against caged adult *Aedes aegypti*. *J Am Mosq Control Ass*, 31(1):85-92.
- Derraik JG, Slaney D. 2015. Notes on Zika- an emerging pathogen now present in the South Pacific. *Aust N Z J Public Health* 39:5-7.
- Dias C, Moraes D. 2013. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasite Research*, 113:565-592.
- Doyle MJ, Kline DL, Allan SA, Kaufman PE. 2009. Efficacy of residual bifenthrin applied to landscape vegetation against *Aedes albopictus*. *J Am Mosq Control Ass* 25:179-183.
- Fulcher A, Farooq M, Smith, ML, Li CX, Scott JM, Thomson E, Kaufman PE, Xue RD. 2015. Evaluation of a new spraying machine for barrier treatment and penetration of bifenthrin on vegetation against mosquitoes. *J Am Mosq Control Ass*, 3(1):85-92.
- Gerberg EJ, Barnard DR, Ward RA. 1994. Manual for mosquito rearing and experimental techniques. *AMCA Bulletin* 5. Lawrence, KS: Allen Press.
- Gross AD, Coats JR. 2015. Can green chemistry provide effective repellents? *Insect Repellents Handbook* (ed. M. Debboun, S.P. Frances, D.A. Strickman), pp. 75-90. CRC Press and Taylor and Francis Group LLC, Boca Raton, FL.
- Gross AD, Norris EJ, Kimber MJ, Bartholomay LC, Coats JR. 2017. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. *Med Vet Entomol*, 34:55-62
- Manica M, Cobre P, Rosà R, Caputo B. 2016. Not in my backyard: effectiveness of outdoor residual spraying from hand-held sprayers against the mosquito *Aedes albopictus* in Rome, Italy. *Pest Manag Sci*, 73, 138-145.
- Montefuscoli, A, J. Werdin-Gonzlez, S. Palma, A. Ferrero, and B. Fernandez. 2013. Design and develop-

- ment of aqueous nanoformulations for mosquito control. *Parasit Research*, 113:793-800.
- Mulla MS, Norland LR, Fanara DM, Darwezeh HA, McKean DW. 1971. Control of chironomid midges in recreational lakes. *J Econ Entomol*, 64:300-307.
- Norris EJ, Gross AD, Dunphy B, Bartholomay LC, Coats JR. 2015. Comparison of the insecticidal characteristics of commercially available plant essential oils against *Aedes aegypti* and *Anopheles gambiae*. *J Med Entomol*, 52:993-1002.
- Tong F, Bloomquist JR. 2013. Plant essential oils affect the toxicities of carbaryl and permethrin against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*, 50(4):823-832.
- Wilson ME, Chen LH. 2016. Dengue: update on epidemiology. *Curr Infect Dis Rep* 17:457.
- World Health Organization (WHO). (2017a) *Vector-Borne Diseases*. Retrieved May 11th, 2018, from <http://www.who.int/mediacentre/factsheets/fs387/en/>
- World Health Organization (WHO). (2017b) *Chikungunya*. Retrieved July 15<sup>th</sup>, 2019, from <https://www.who.int/news-room/fact-sheets/detail/chikungunya>
- World Health Organization (WHO). (2017c) *Dengue and severe dengue*. Retrieved July 15<sup>th</sup>, 2019, from <https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue>
- Xue RD. 2008. Toxicity of permethrin-, malathion-, and fipronil-treated plant foliage to *Aedes albopictus* and *Aedes aegypti*. *J Am Mosq Control Ass*, 24(1):169-171.

# SEMI-FIELD ULV EVALUATION OF AN ALL-PURPOSE BOTANICAL INSECTICIDE CONTAINING CEDARWOOD AND CINNAMON OILS AGAINST ADULT *Aedes aegypti*

CHRISTOPHER S. BIBBS, KATHY SHIRLEY, DENA L. AUTRY,  
AND RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Subject editor: Eva Buckner

## ABSTRACT

Public health mosquito control operates with only two classes of mosquito adulticides: pyrethroids and organophosphates. Recent work improving the emulsification of essential oils has increased the potential for development of plant-derived active ingredients. There is a growing body of literature on essential oils for various roles in mosquito management. NatureCide Pest Management (NCPM), a product available in private and commercial home pest control, uses a mixture of 25.3% cedarwood oil and 12.7% cinnamon oil as a Federal Insecticide, Fungicide, Rodenticide Act (FIFRA) exempt insecticide for both indoor and outdoor use. Recent investigations by the Anastasia Mosquito Control District of St. Johns County have found other FIFRA exempt products to be effective as a residual spray on vegetation. In continuing the exploration of botanical insecticides, NCPM was used in ULV tests against *Aedes aegypti* (L.) within its 35-122 ml per L of water label rate. Applications at 35 ml/L resulted in 60-70% knockdown after 1 hr and mortality after 24 hr. Increasing the rate to 70 ml/L resulted in 100% knockdown and mortality across all replications. Crystalline precipitation of the microemulsion was observed in mix tanks after standing for at least 2 wk, but it was not apparent that the efficacy of the product was reduced as a consequence. Cedarwood oil and cinnamon oil are a beneficial combination for ULV adulticiding against mosquitoes and could have a beneficial role for integrated mosquito management.

Key Words: *Aedes aegypti*, mosquito, botanical, insecticide, essential oils

## INTRODUCTION

Botanical ingredients are attractive alternatives in formulated repellents (Gross and Coats 2015), toxicants (Gross et al. 2017), and synergists (Tong and Bloomquist 2013; Gross et al. 2017). The sustained demand for plant-derived active ingredients in pesticides has prompted the screening of over 350 plant essential oils as larvicides against *Aedes aegypti* (L.) (Dias & Moraes 2013). Phytochemicals have become increasingly viable for product development since successful formulation in microemulsions (Gross et al. 2017), and microemulsion formulations were demonstrated in pilot work as effective against *Culex pipiens* (Montefuscoli et al. 2013). In consequence, essential oils also are being screened as adulticides against *Ae. aegypti* and *An. gambiae* (Norris et al. 2015). Despite this effort, few products exist for mosquito management that use plant-derived active-ingredients, par-

ticularly for ultra-low volume (ULV) cold aerosol space sprays.

Amidst the emphasis on green chemistry underlies the principle cause of the demand for this research: EPA allows minimum risk pesticides to be exempt from FIFRA (40 C.F.R. §152.25 2015). This exemption is ideal for green products because environmental impact is minimal, and the product may be used more frequently than a FIFRA labeled product. This fundamentally appeals to desires for reapplication treatments when managing a significant mosquito outbreak or when mitigating arbovirus transmission. Furthermore, mosquito control is currently limited to two chemical classes for adulticides, which are the FIFRA regulated pyrethroids and organophosphates. However, exempt pesticides would provide different active ingredients for minimizing both resistance and environmental impacts.

One example of an exempt product, NatureCide Pest Management (NCPM), uses 25.3% cedarwood oil and 12.7% cin-

namon oil as active ingredients. Cedarwood oil has been explored as a repellent against mosquitoes, ticks, and ants (Khanna and Chakraborty 2018; Eller et al. 2014), but has consistently shown high proclivity for killing arthropods, especially public health pests (Khanna and Chakraborty 2018; Eller et al. 2014; Singh et al. 1984). Cinnamon oil is an octopaminergic insecticide (Kostyukovsky et al. 2002) that expressed the greatest toxicity of eight adulticidal essential oils screened against adult *Culex quinquefasciatus* (Say) and *Musca domestica* (L.) (Benelli et al. 2018). It is also a synergist that increases the bioefficacy of other essential oils when presented together (Reegan et al. 2014).

The cedar and cinnamon oil mixture of NCPM is labeled for use against a variety of indoor and outdoor pests, including ants, fleas, filth flies, and other arthropods. Both of the aforementioned NatureCide products are not labeled for use as a space spray, instead being prescribed at rates for outdoor residual sprays. There is limited exploratory work with this and similar commercial products. However, utilization as a cold aerosol for ULV would provide more options to mosquito control. Therefore, we tested NCPM, which was recommended by the manufacturer for mosquito management, at the low end of its label rate to help determine the ULV potential of this alternative tool.

## MATERIALS AND METHODS

The mosquito strain selected for testing was the 1952 Orlando strain *Aedes aegypti* sourced from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology and reared in the insectaries of the Anastasia Mosquito Control District of St. Johns County. Mosquitoes were maintained at  $26 \pm 1.0^\circ\text{C}$ , 65-80% relative humidity, and a photoperiod of 14:10 hr (L:D). The adult mosquitoes were provided 10% sugar solution as needed. Once mosquitoes were 5-7 d old, non-blood-fed females were selected for testing. To conduct assays, twenty

females were transferred into cylindrical screened cages (4 x 10 cm) with the use of a HEPA-filtered mouth aspirator. Caged mosquitoes were acclimated to outdoor conditions for a minimum of 20 min prior to the start of any applications.

Treatments were carried out using NatureCide Pest Management (25.3% Cedarwood oil, 12.7% cinnamon oil, Pacific Shore Holdings, Inc., Canoga Park, CA). The label prescribed recommendation was to mix the product at a range of 35-122 ml per liter of water. For these tests, dilutions were arbitrarily selected at 35 ml/L and 70 ml/L. The formulation was applied by a truck-mounted single nozzle ULV cold aerosol sprayer (Guardian 95 ES, ADAPCO, LLC, Sanford, FL). The machine was calibrated to dispense droplets with an average size of 18 microns, spanning VMD of 10-30 microns ( $10 \mu \leq Dv \leq 30 \mu$ ), at 296 ml/min (10 oz/min). For each treatment, a row of polyvinyl chloride pipe stands, 1.2 m in height, held the mosquito cages mounted at 0.8-1 m above ground level. Stands were placed in three equidistant rows approximately 30 m, 60 m, and 90 m downwind from the truck drive path. Tests were conducted in the morning (0700 h-1100 h), with wind direction, wind speed, temperature, and relative humidity recorded on site. Spray trucks were driven at an average of 16 kilometers per hour in a straight line perpendicular to the length of the hanging field cage line. The treatment started 30 m prior to the first pipe stand and the treatment was shut off at 30 m past the last stand to ensure coverage during variable wind conditions. After the treatment, 15 min was allowed for drift to ensure passage of the spray plume downrange past the test plot before cages were gathered and returned to the laboratory for processing. Both dilutions were evaluated across three replications each. Once returned to the laboratory, mosquitoes were provided with 10% sucrose solution (in water) overnight using saturated cotton balls. Knockdown was recorded at 1 h and mortality was recorded 24 h post-treatment. Sets of 3 control cages per replicate



were handled in an identical manner except being placed 30m upwind of the truck during application.

Data was corrected for control mortality below 10% by using Abbott's formula (Abbott 1925). Variation between field tested dilutions were analyzed in JMP 13.1.0 (SAS Institute, Inc., Cary, NC) using analysis of variance (ANOVA) and Tukey HSD test.

### RESULTS AND DISCUSSION

Weather conditions averaged 27.5°C air temperature, 77.2% RH, and persistent south-southwest wind direction at 3.7 km/

hr. Day conditions were clear and sunny with no persistent cloud cover or precipitation. Field assay data are summarized with mean and standard error of the mean (SEM) knockdown and mortality rates provided for 35 ml/L and 70 ml/L of NCPM in Fig. 1. There were no significant differences between the position in the 3 x 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ( $F_{2,26} = 1.278, p = 0.3072$ ) or mortality ( $F_{2,26} = 2.4967, P = 0.1159$ ) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications ( $p < \text{no variance}$ ). The lowest rate, 35 ml/L, averaged 60-70% knockdown

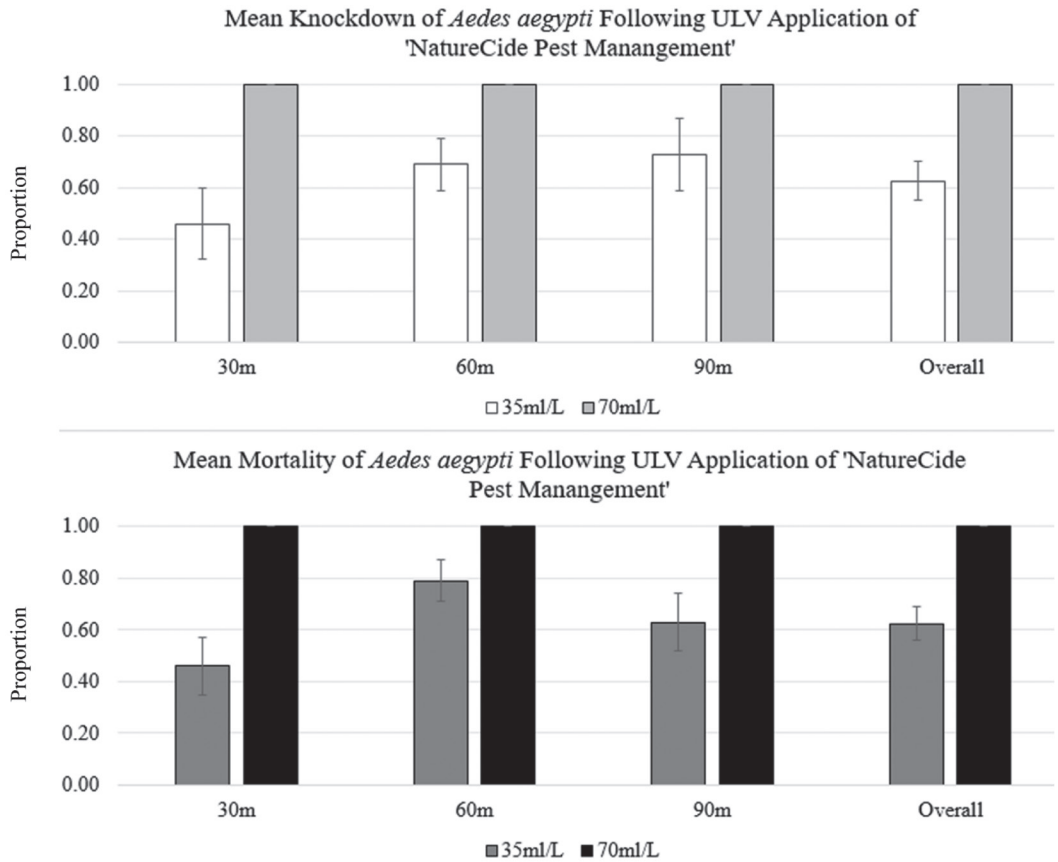


Figure 1. Significant mean (+ SEM) 1 hr knockdown and 24 hr mortality of *Aedes aegypti* (L.) were observed following ULV treatment with 35 ml/L and 70 ml/L of NatureCide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) in a 3 x 3 grid with 30 m equidistant separations between mosquito cages ( $F = 5.34, df = 5, 54, p < 0.0005$ ). There were no significant differences between the position in the 3 x 3 test array nor at discrete distances (30m, 60m, 90m) within knockdown ( $F_{2,26} = 1.278, p = 0.3072$ ) or mortality ( $F_{2,26} = 2.4967, P = 0.1159$ ) for 35ml/L. Treatments made at 70ml/L resulted in 100% knockdown and mortality at all distances and all replications ( $p < \text{no variance}$ ). Treatments with 35 ml/L and 70 ml/L fell inside the low end of the label allowed rates of 35-122ml per liter of water.

and mortality among the exposed *Ae. aegypti* (Fig. 1). In contrast, all values for 70 ml/L were 100% for knockdown and mortality regardless of distance or position (Fig. 1). Knockdown and mortality were significantly greater at 70 ml/L than 35 ml/L, which was significantly greater than observed in the controls ( $F = 5.34$ ,  $df = 5$ ,  $54$ ,  $p < 0.0005$ ). Control mortality was 0% in all trials.

Unexpectedly, crystalline precipitates were found on the surface of the liquid (Fig. 2) in the mix tank after the solution aged on the truck ULV assembly for 2 wk. Replicates using precipitated mixture were omitted from the data analysis, however there did not appear to be an obvious toxicity change when using precipitated mixtures. Freshly diluted product was used for each replicate and mix tanks were held for 6 wk after use. The crystalline precipitation occurred in all mixes regardless of

which dilution. Agitation did not appear to resolve the precipitation of aged mixtures. Precipitation did not occur when mixtures were kept in cooler, laboratory conditions.

We intended to test farther into the label range for NCPM, however it was surprising to see it was not necessary to go higher than 70 ml/L, and perhaps not even necessary to go much higher than 35 ml/L. We did not test larvicide potential in this study, but it is also possible that exempt products made from botanical ingredients may be equally useful for larvicides as they are for adulticides (Norris et al. 2015). Furthermore, there may be additional benefits of NCPM in broader integrated management questions. Several examples of botanical oils for mosquito control are functional as repellents (Gross and Coats 2015) or synergists (Tong and Bloomquist 2013; Gross et al. 2017). Intensive screening of 361 essential oils from 269 plant spe-

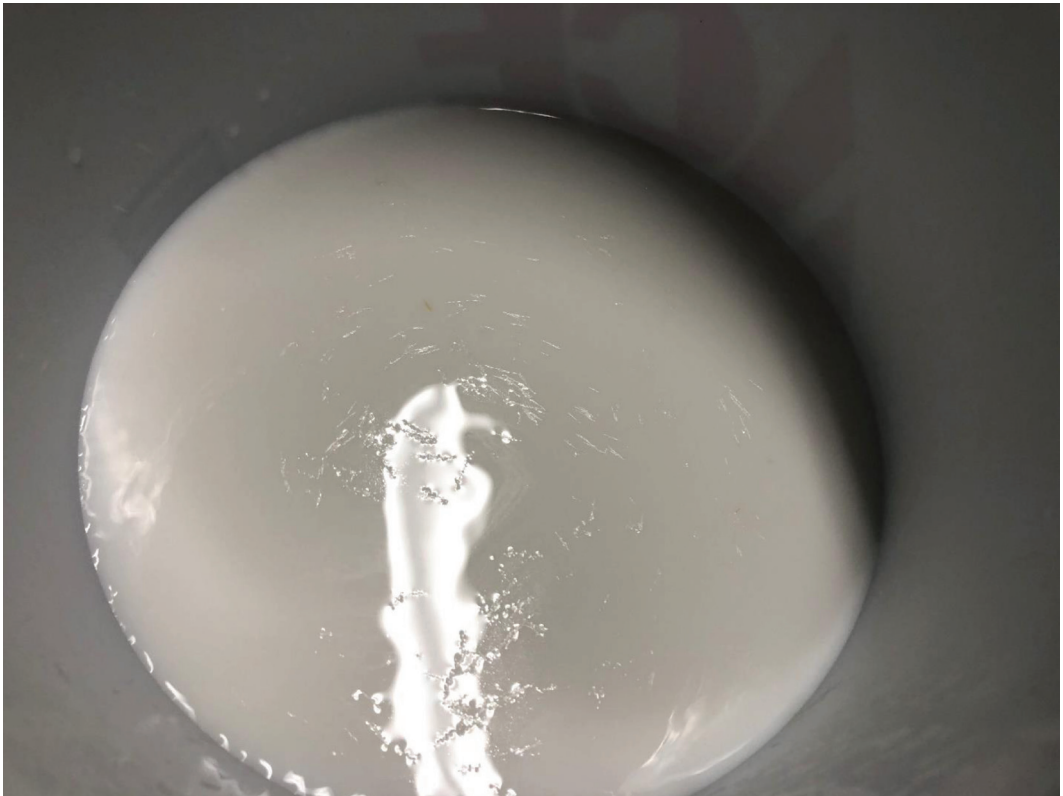


Figure 2. Crystalline precipitation in the mix tank for NatureGide Pest Management (25.3% cedarwood oil, 12.7% cinnamon oil) after 2 wk of storage after a replicate of truck mounted ultra-low volume cold aerosol treatment. Mixtures were left on the truck between the conclusion of treatment and the time of this image.

cies revealed dozens of potential larvicides against *Ae. aegypti* (Dias & Moraes 2013). Despite the aforementioned evidence, mosquito control has been slow to acquire botanical products for residual treatments or ultra-low volume (ULV) cold aerosol space sprays. By translating NatureCide Pest Management or similar products into public health operations, mosquito control can gain wider access to “green” alternative adulticides that do not have reapplication restrictions.

NatureCide Pest Management an EPA exempt product currently labeled for indoor and outdoor residual spot treatments against an assortment of urban and peridomestic insect pests. Meanwhile, the active ingredients, essential oils, appeal to eco-friendly proponents of botanical insecticides while still presenting a potentially effective mosquito adulticide. There may be broader utility in using these products if it also expands the circumstances or land area in which intervention can be made to reduce mosquitoes. The success of micro-emulsion formulations appears to be one reason that products may become more available from the discovered bioactive essential oils (Montefuscoli et al. 2013, Gross et al. 2017). Other FIFRA exempt products also have shown high comparative efficacy. Evaluation of an exempt sister product, NatureCide All-Purpose Commercial Concentrate containing clove and cottonseed oil, showed that when used as a vegetative barrier spray it outperformed Essentria IC<sup>3</sup> (rosemary oil, peppermint oil), Onslaught (fenvalerate), DeltaGard (deltamethrin), and performed equivalently with Cyzmic (lambda-cyhalothrin) (Smoleroff et al. 2019).

However, the stability of the micro-emulsions is not well understood in an operational context. The precipitation we observed in the mix tanks may imply that precautions need to be made with NCPM or similar essential oil emulsions if incorporating them into the machinery used in mosquito control operations. As an additional consideration, understanding non-target effects may in turn facilitate expansion

of the label and trust in the blend of active ingredients in NCPM and similar products. Given the exemption status and consequent potential to reapply this insecticide frequently, it is critical to understand the non-target impacts of application on key pollinators or to water ecology. Regardless of the gaps in knowledge, we believe our positive results using NatureCide Pest Management as a ULV treatment highlights that some botanicals are ready to be incorporated into mosquito control programs.

#### ACKNOWLEDGEMENTS

We would like to thank Jason Conrad and Univar for their assistance with selecting and sourcing the product chosen for this study. This is a research report only. Specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District.

#### REFERENCES CITED

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol*, 18: 265-267.
- Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, Petrelli R, Maggi F. 2018. Acute and sub-lethal toxicity of eight essential oils of commercial interest against filiaris mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. *Ind Crops Prod*, 112: 668-680.
- Dias C, Moraes D. 2013. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. *Parasit Research*, 113: 565-592.
- Eller FJ, Vander Meer RK, Behle RW, Flor-Weiler LB, Palmquist, DE. 2014. Bioactivity of cedarwood oil and cedrol against arthropod pests. *Environ Entomol*, 43: 762-766.
- Gross A D, Coat JR. 2015. Can green chemistry provide effective repellents? *Insect Repellents Handbook* (ed. by M. Debboun, S.P. Frances & D.A. Strickman), pp. 75-90. CRC Press and Taylor and Francis Group LLC, Boca Raton, FL
- Gross AD, Norris EJ, Kimber MJ, Bartholomay LC, Coats JR. 2017. Essential oils enhance the toxicity of permethrin against *Aedes aegypti* and *Anopheles gambiae*. *Med Vet Entomol*, 34: 55-62.
- Khanna S. Chakraborty JN. 2018. Mosquito repellent activity of cotton functionalized with inclusion complexes of  $\beta$ -cyclodextrin citrate and essential oils. *Fashion Textiles*, 5: 9.
- Kostyukovsky M, Rafaely A, Gileadi, C. Demchenko N, Shaaya E. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. *Pest Manag Sci*, 58: 1101-1106.

- Montefuscoli A, Weiridin-Gonzlez J, Palma S, Ferrero A, Fernandez B. 2013. Design and development of aqueous nanoformulations for mosquito control. *Parasit Research*, 113: 793-800.
- Norris E J, Gross AD, Dunphy B, Bartholomay LC, Coats JR. 2015. Comparison of the insecticidal characteristics of commercially available plant essential oils against *Aedes aegypti* and *Anopheles gambiae*. *J Med Entomol*, 52: 993-1002.
- Reegan A D, Kannan RV, Paulraj MG, Ignacimuthu S. 2014. Synergistic effects of essential oil-based cream formulations against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). *J Asia-Pacific Ent*, 17: 327-331.
- Singh D, Rao SM, Tripathi AK. 1984. Cedarwood oil as a potential insecticidal agent against mosquitoes. *Naturewissenschaften* 71: 265-266.
- Smoleroff ST, Bibbs CS, Xue RD. 2019. Evaluation of pyrethroid and botanical barrier insecticides against *Aedes albopictus* in the laboratory and field. *J. Florida Mosq Control Assoc* 66:
- Tong F, Bloomquist JR. 2013. Plant essential oils affect the toxicities of carbaryl and permethrin against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*, 50: 826-832.

# OPERATIONS NOTE & EQUIPMENT

## STORE TIME OF PERMETHRIN IN THE TRUCK-MOUNTED ULV SPRAY TANKS CAUSED DEGRADATION IN THE CONCENTRATIONS

RUI-DE XUE<sup>1</sup>, MAIA TSIKOLIA<sup>2</sup>, WHITNEY A. QUALLS<sup>3</sup>,  
AND ULRICH BERNIER<sup>2</sup>

<sup>1</sup>Anastasia Mosquito Control District, 120 EOC Drive,  
St. Augustine, FL, 32092.

<sup>2</sup>USDA/ARS, Center for Medical, Agriculture, and Veterinary Entomology,  
Gainesville, FL 32608

<sup>3</sup>Texas Department of State Health Service/ Zoonosis Control Branch,  
Austin, TX 78714

Guest Editor: Caroline Efstathion

### ABSTRACT

Permethrin is one of the few active ingredients found in commercial adulticides available for adult mosquito control. The permethrin concentrations in the adulticide Aqualuer 20-20 formulation stored in the 21 ultra-low-volume (ULV) truck-mounted spray tanks after 4, 6, and 8 months were sampled and analyzed by gas chromatography mass spectroscopy (GC-MS). Additionally, the permethrin stored in a mixing pump and a stock container were sampled and analyzed by GC-MS. The results showed that 46%, 42%, and 82% permethrin in Aqualuer 20-20 were decomposed in the ULV spray tanks at 4-, 6-, and 8 months-storage, respectively. For the mixing pump, 17% of the permethrin in the Aqualuer 20-20 were decomposed. The storage time of permethrin in ULV spray tanks resulted in the degradation in the concentrations of permethrin after more 4 months, compared with the concentration in stock control. The degradation of permethrin in ULV spray tanks may directly impact the control efficacy for adult mosquitoes and result in an economic loss.

Key Words: permethrin, decomposition, adulticide, Aqualuer, storage time, gas chromatography mass spectroscopy (GC-MS), analysis

Permethrin is the active ingredient of several commercial mosquito adulticide products (Amoo et al. 2012; Brown & Xue 2011). Xue et al. 2008 found that about 50% permethrin in the adulticide previously used by the mosquito control program decomposed in the truck-mounted ultra-low-volume (ULV) spray tanks when mixed with water and stored over the winter for the upcoming mosquito season. However, we do not know whether time of storage impacts the degradation of permethrin after mixing with water. The purpose of the study was to examine whether time, 4, 6, and 8 months, of storage of the mixed permethrin product

Aqualuer 20-20 (active ingredient is 20.6% permethrin, AllPro Inc., P. O. Box 585, St. Joseph, MO 64502) in the truck-mounted ULV spray tanks and in the mixing pump impacts the permethrin concentration.

The assessment was conducted by taking permethrin samples from each spray tank (total 21 trucks) and one mixing pump. The sampling process included agitating the mixture for 5 mins with a handled agitator before taking the sample from each tank. A 10- mL sample from the middle of the spray tank was taken with a 10-mL disposable plastic pipette. The samples were immediately placed into 20 mL glass bottles and

labeled according to the truck tank numbers and date. The water-mixed solution from the mixing pump was drained to a plastic bucket and a 10 mL solution was sampled from the bucket after agitation. Control samples were obtained from the stock container following the agitation after circulation and dilution 1:10 Aqualuer 20-20 and well water. All samples were sealed, stored with coolants, and transferred for analysis to the United States Department of Agriculture (USDA), Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Gainesville, FL.

All samples were analyzed for permethrin residue by using a gas-chromatography mass spectroscopy (GC-MS). Permethrin (98.0% purity) was purchased from Chem Service, Inc., West Chester, PA; Internal standard (ISTD) dibutyl phthalate (99% purity) from Supelco, Bellefonte, PA. Quechers SPE Resprep 15 mL tubes with 900 mg MgSO<sub>4</sub>, 300 mg PSA (primary and secondary amine exchange material), and 150 mg GCB (graphitized carbon black) were purchased from Restek, Bellefonte, PA. Acetone (HPLC grade) was obtained from Sigma-Aldrich, Saint Louis, USA.

Pyrex conical-bottom 15 ml disposable glass centrifuge tubes (Cole-Parmer North America, 625 East Bunker Court, Vernon Hills, IL 60061, USA) were used for sample preparation. Sample solutions were transferred using Fisher brand disposable borosilicate glass Pasteur pipets (purchased from Sigma-Aldrich, Saint Louis, USA), 9-inch pipets were used to transfer the sample solutions and 5.75-inch pipets to collect concentrated solution through cotton filter into the 1 mL Pyrex cylindrical volumetric flask with stopper #8 (Fisher Scientific, Waltham, MA, USA). Five- and eight mL amber bottles were used to store stock and internal standard solutions. For sonication, shaking, vortexing and centrifuging the following instruments were used: Model FS140 Sonic Bath, Genie 2 Vortex mixer 12-812 and Centrifuge Model 228 (all from Fisher Scientific, Waltham, MA, USA), Excella E2 Platform shaker (New Brunswick Scientific, Enfield, CT, USA).

Three stock standard solutions were prepared as follows: Three 20 mL blank samples were transferred into Quencher's 15 ml centrifuge tubes, each spiked with 2.5 mg permethrin, added acetone up to 6 mL, vortexed (30 sec), sonicated (30 min), shake (15 min, 300 rpm), centrifuged (15 min, 600 rpm) and transferred into glass 15 ml conic centrifuge tube. This procedure was repeated two more times with sonicating 15- and 5 min, respectively. Then the collected sample solution in glass conic centrifuge tube was concentrated to 0.5 ml. The concentrated solution was transferred into 1 mL volumetric flask through 0.5 mg cotton filter (placed in the 5.75-inch Pasteur pipet). The centrifuge tube was washed twice with acetone and the solution was added to a volumetric flask. The solution in volumetric flask was filled up to 1 mL with acetone. Then, each recovered standard stock solution was diluted accordingly to yield solutions of five different concentrations for each range: 1st: 20-100 ng/mL (20, 50, 70, 80 and 100 ng/mL), 2nd: 200-500 ng/mL (200, 250, 300, 400 and 500 ng/mL) and 3rd: 600-1,000 ng/mL (600, 700, 800, 900 and 1000 ng/mL). A 10 mL aliquot of 1,000 ng/mL ISTD-dibutyl phthalate solution was added to each diluted pyrethroid stock solution using a micro syringe. All stock solutions were stored in a freezer at -20 °C. Standard solutions were analyzed immediately after preparation followed by sample analysis.

The sample (20 mL) was transferred into Quechers 15 ml centrifuge tube, then 3 mL of acetone was added and vortexed (30 sec), sonicated (30 min), shake (15 min, 300 rpm), centrifuged (15 min, 600 rpm) and transferred into 15 mL glass conic centrifuge tubes. This procedure was repeated two more times, sonicating 15- and 5 min, respectively. Then collected sample solution in glass conic centrifuge tube was concentrated to 0.5 mL. The concentrated solution was transferred into 1 mL volumetric flask through 0.5 mg cotton placed in the 5.75-inch Pasteur pipets to the volumetric flask and acetone was added until the final volume reached 1 mL. Then

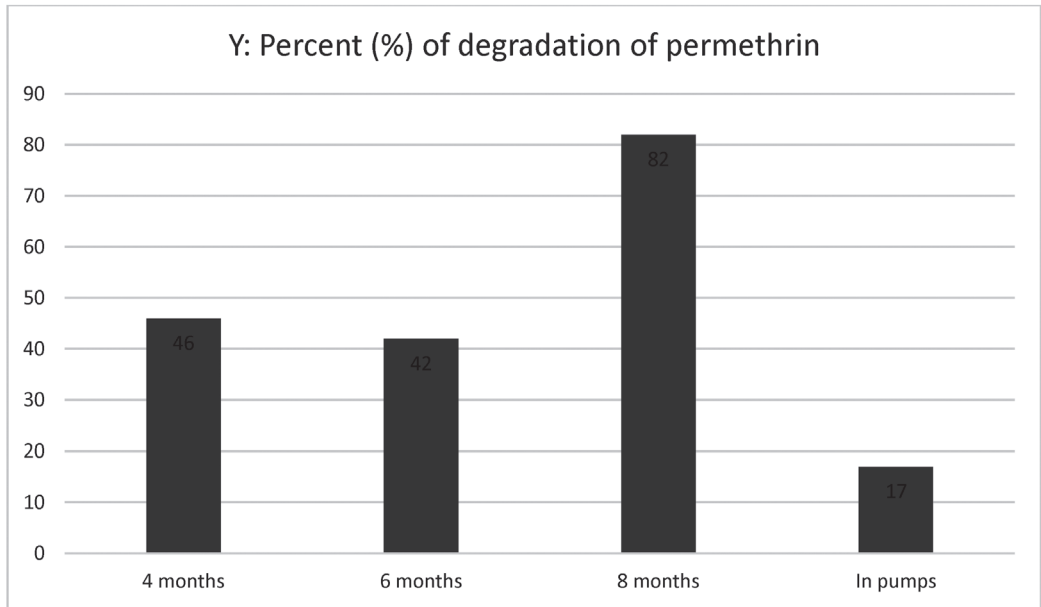


Figure 1. Store time (in months) of Aqualuer 20-20 (a.i. 20.6% permethrin) and degradations (in %) of permethrin concentrations in truck-mounted ULV spray tanks after mixed with water for different months, compared with concentrations in stock Aqualuer 20-20 container (as zero degradation).

5 mL solution from the volumetric flask was transferred into GC/MS amber vial 10 mL of ISTD solution (1,000 ng/mL) and, then acetone was added until the final volume reached 1 mL. This procedure was performed for all 21 samples. Samples were then subjected to the GC-MS analysis immediately after preparation.

A 1 $\mu$ L aliquot from each extract was analyzed along with the appropriate solvent blanks using a Thermo Finnigan DSQ (Thermo Fisher Scientific; Austin, TX, USA) equipped with a DB-5 (Agilent; Santa Clara, CA, USA) column (30 m  $\times$  0.25 mm inner diameter and 0.25  $\mu$ m the film thickness). The GC oven temperature program was set at 100 °C as initial temperature, then a ramp at 16 °C/min to 200 °C, and at 7 °C/min to 300 °C followed by a final hold for 5 min at 300 °C. The PTV injection port was held at 50 °C and ramped to 240 °C in split less mode, the transfer line was set to 240 °C, and the carrier gas was set to a constant flow of 1.2 mL/min. Analytical mode was set on Selected Ion Monitoring (SIM). The ions selected for SIM quantification of the permethrin were 163 and 183.

During the validation of the analytical method, linear range, limit of detection (LOD), limit of quantification (LOQ), and precision were determined. Linearity was evaluated with 5-point linear plot (three replicates), based on linear regression and squared correlation coefficient  $R^2 > 0.990$ . Standards were prepared by spiking a blank with permethrin and then subjecting to the same treatment, as samples. Precision of method was expressed as standard deviation (SD) (three replicates). Precision of the instrument was expressed as the repeatability of the measurements (SD, 6 replicates at concentrations 50, 100 and 250 m/L). LOD and LOQ were calculated based on the noise level in the chromatograms at S/N of 3:1 and 10:1 correspondingly. Each sample identification number with store time in months was sorted to a group of 4 months, 6 months, and 8 months. The permethrin degradation (%) in concentration of each sample was calibrated by using the concentration (X) in stock container minus the concentration (Y) in sample (X-Y) divide the concentration in stock (X), then multiple 100%. A one-way ANOVA was performed for the data analysis for the percentage of degra-

dation of permethrin through JMP 13.10 (SAS Institute, Inc. Cary, NC).

A total of 21 truck-mounted ULV spray tanks, one mixing pump, and one stock container were sampled and analyzed by gas chromatography using the methods described above. Two of the 21 samples from truck spray tanks showed 0% permethrin after analysis. These trucks were designated for fogging only at night and early morning. This result was probably caused by a malfunction with the mixing pump when adding the insecticide or other unknown reasons. Two dual duty trucks (larviciding and adulticiding operated at daytime and nighttime) showed 93%-98% permethrin degradation. This may be a result of exposure to sunlight and heat during the daytime outdoor use (Amoo et al 2008). All other samples showed significantly ( $F = 25,255$ ,  $P < 0.01$ ) different concentrations of permethrin. Figure 1 shows that 17% degradation in mixing pump with water, and the average of 46%, 42%, and 82% degradation of permethrin in concentrations stored in ULV spray tanks for 4, 6, and 8 months, during the mosquito off season, compared with the concentrations sampled from the stock container. The percent degradation between 4 months (46%) and 6 months (42%) did not show any significant differences. The permethrin concentration after storage for 8 months or more demonstrated ( $F = 23,407$ ,  $P < 0.01$ ) the highest degradation after mixing with water.

In conclusion, the longer the storage time (in months) of permethrin (after being mixed with water during the mosquito off season) in truck-mounted ULV spray tanks, the more degraded it became. For the improvement of operational control effectiveness, the concentrations of permethrin in spray tanks, if stored over the mosquito off season, should be analyzed before conducting ULV spray and after the product has been mixed with water for several months. If low concentrations of the active ingredient permethrin are detected, additional permethrin should be added from the stock container. Also, emptying spray tanks after the mosquito season or adding new mixed permethrin into the spray tank before conducting ULV spray is recommended.

#### REFERENCES CITED

- Amoo AO, Xue RD, Qualls WA, Quinn BP, Bernier UR. 2008. Residual efficacy of field-applied permethrin, d-phenothrin, and resmethrin on plant foliage against adult mosquitoes. *J. Am. Mosq. Control Assoc.* 24:543-549.
- Amoo AO, Xue RD, Qualls WA, Wang ZM, Zhao TY. 2012. Efficacy evaluation of three permethrin products against caged *Culex quinquefasciatus*. *Acta Parasitol Med. Entomol. Sinica* 19:19-24.
- Brown JR, Xue, RD. 2011. Ground application of Aqueslin and Aquakontrol against *Anopheles quadrimaculatus*, *Aedes aegypti*, and *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 27:330-332.
- Xue RD, Qualls WA, Zhong H, Brock CL. 2008. Permethrin decomposition after four month storage in the spray truck tanks during mosquito-off season. *J. Am. Mosq. Control Assoc.* 24:127-129.



# EVALUATION OF CDC LIGHT TRAP, BG SENTINEL TRAP, AND MMX TRAP FOR THE COLLECTION OF SALT MARSH MOSQUITOES IN ANASTASIA STATE PARK, SAINT AUGUSTINE, FLORIDA

DANIEL DIXON<sup>1</sup>, JOSEPH D'AMATO, AND RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, Saint Augustine, FL 32092

Current address: USDA/CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608

Guest Editor: Emad Khater

## ABSTRACT

Salt marsh mosquitoes are major nuisance pests during the periods of high mosquito activity, especially after major storm events. In 2016-2017, Saint John's County, Florida, USA was struck by two major hurricanes that resulted in multiple outbreaks of salt marsh mosquito populations. To optimize the surveillance of two salt marsh mosquitoes, (*Aedes taeniorhynchus* and *Ae. sollicitans*, three types of traps (the Centers for Disease Control (CDC) Light trap, Biogents Sentinel (BG) trap and Counter Flow Geometry Model (MMX) trap were tested for their capacity to capture the highest numbers of high quality live specimens for laboratory bioassays. Each trap type was tested in Anastasia State Park, located along a major salt marsh area in Saint Johns County. Although the MMX trap captured most of the salt marsh mosquitoes collected, the number of mosquitoes captured was not statistically significant compared to the other trap types. However, there was a significant difference in the numbers between *Ae. taeniorhynchus* and *Ae. sollicitans* in the MMX traps.

Key Words: *Aedes taeniorhynchus*, *Aedes sollicitans*, surveillance, salt marsh, CDC light trap

Salt marsh mosquitoes are nuisance pests to Florida residents and tourists due to their aggressive biting behavior and long flight range (Rey et al. 2012; Nayar 1985; Hribar et al. 2010). These mosquitoes are also of a public health concern to people due to allergic reactions caused by their bites and their potential as vectors of disease pathogens such as the dog heartworm and Venezuelan Eastern Encephalitis virus, both to dogs and residents in Saint Johns County, Florida, USA, respectively (Peng et al. 2004; Sudia et al. 1971; Weaver et al. 1996). It was noted that seasonality, temperature, flood conditions and hurricanes are the major factors that affect egg hatching rates, which result in noticeable increases in populations of salt marsh mosquitoes (Hribar et al. 2010).

Two salt marsh mosquito species, *Aedes taeniorhynchus* (Weidemann) and *Ae. sollicitans* (Walker) are primarily targeted by the Anastasia Mosquito Control District (AMCD), St. Augustine, FL to control. *Aedes Taeniorhynchus*,

also known as the Black Salt Marsh mosquito, breeds in brackish water in North, Central, and South America; while *Ae. sollicitans*, also known as the Eastern Salt Marsh mosquito, is found in saline wetlands, dense salt marshes, shallow pools, and inland in collected brackish water. In the 2016-2017, Saint Johns County was hit by thunderstorms throughout the summer and two hurricanes (Matthew and Irma) that accounted for over 56 cm of rainfall. Over 200 service requests per day were raised from the residents living near salt marsh habitats on Anastasia Island. Accordingly, and in prompt response, AMCD sent multiple teams of technicians to treat the island using insecticides, like Mosquito Mist and Aqualuer 20-20 at night and Talstar-P and DUET during the day. However, the repeated and intensive treatments against the salt marsh mosquito populations raised concerns of the potential risk of insecticide resistance development in

the targeted mosquito populations. To test for insecticide resistance in the salt marsh mosquito populations, AMCD used the CDC bottle bioassay, a method that determines the time required for an insecticide to affect

a mosquito from initial exposure to knock-down and then final death.

Optimized trapping protocols are paramount for the CDC bottle bioassay due to the large numbers of intact live mosquitoes

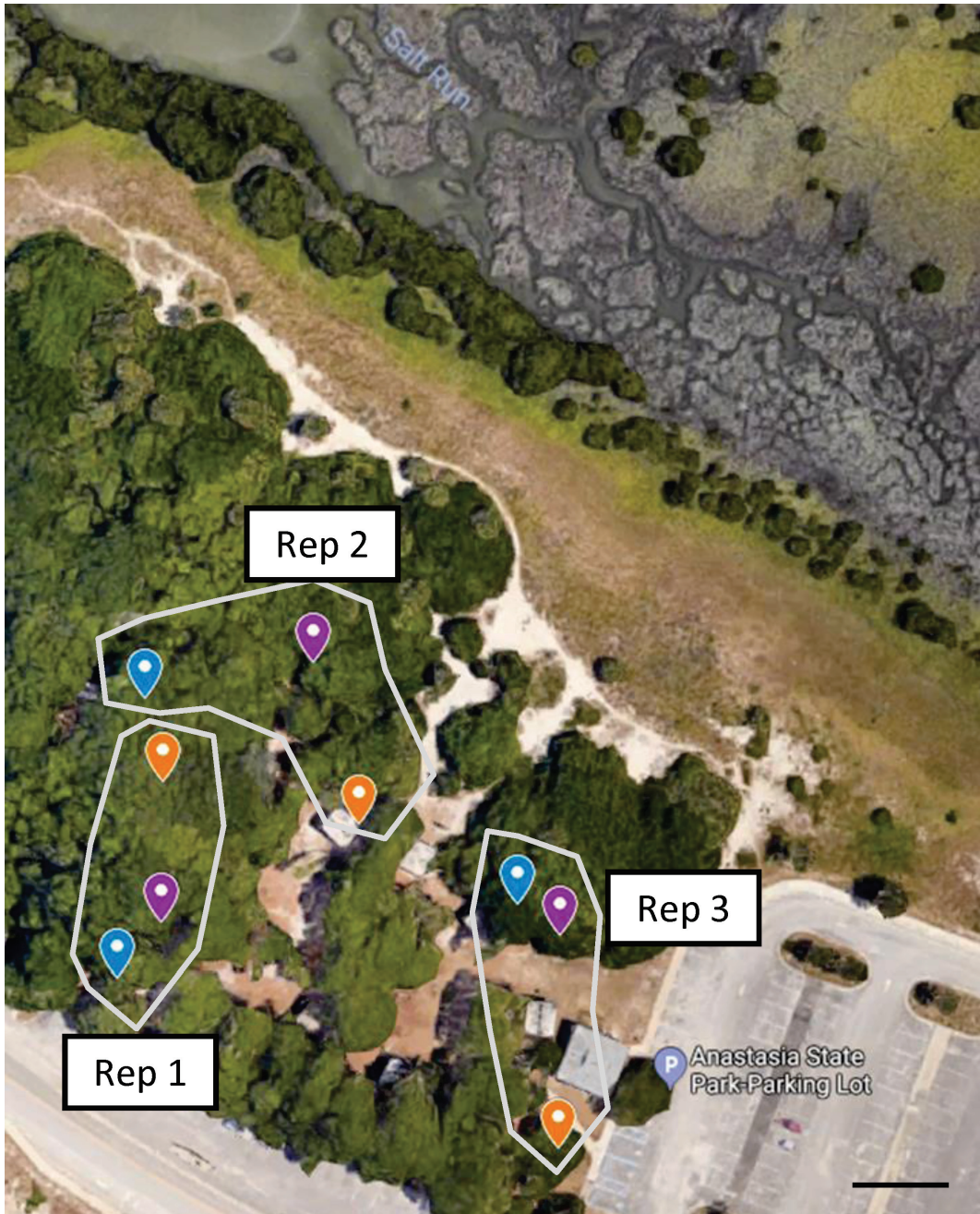


Figure 1. Placement of each MMX trap, CDC light trap, and BG trap. Each replicate (Rep) is encircled by a grey polygon. Within each Rep, the blue blips represent the positions of MMX traps, the purple blips represent the positions of CDC light traps, and the orange blips represent the positions of BG traps. The black scale bar on the right bottom represents 17 meters.

needed to test for susceptibility/resistance bioassays. Most of the previous research has focused on the capture of salt marsh mosquitoes for studying attractant combinations for the CDC light trap (Kline and Lemire, 1995; Rueda et al. 2001). However few studies had been conducted to assess the differences in capture rate of salt marsh mosquitoes by multiple trap types (Smith et al. 2016). Three types of traps, the Centers for Disease Control (CDC) Light trap, Biogents Sentinel (BG) trap and the Counter Flow Geometry Model (MMX) trap were used in this study for salt marsh mosquito collections. This was with the purpose to test the differential capacity of these traps for capturing high quality live salt marsh mosquitoes for lab bioassay.

Three of each trap type were set at a picnic area in Anastasia State Park (29.866186 N, 81.272030 W), and each trap was placed at 9.1–36.6 m apart. All traps were baited with dry ice (carbon dioxide source) as an attractant, but the CDC traps (John W. Hock Company, Gainesville, FL, USA) and BG traps (Biogents AG, Regensburg, Germany) were also baited with Octenol (Biosensory, Putnam, CT, USA) and BG lure (ADAPCO, Sanford, FL, USA), respectively. The MMX traps (American Biophysics Corp., RI) and CDC traps were hung with shepherd’s hooks at one m above ground, while the BG traps were placed on the ground. CDC traps were hooked up to 6-volt batteries; while the BG and MMX traps were hooked up to 12-volt batteries.

All traps were set in the afternoon and collected the next day. All specimens from each trap were identified to species and counted to determine the trap that collected the highest abundance of salt marsh mosquitoes. A goodness-of-fit test was used to determine that the datasets conformed to non-normal, heteroscedastic behavior. Thus,

a non-parametric Kruskal-Wallis test was applied to the data to determine if trap capture differences were statistically significant.

The abundance of the two salt marsh mosquitoes, *Ae. taeniorhynchus* and *Ae. sollicitans* captured in the MMX trap, CDC Light trap, and BG trap was analyzed and presented in Table (1). A total of 697 *Ae. taeniorhynchus* and 154 *Ae. sollicitans*, with an average across all three replicates at 232 *Ae. taeniorhynchus* and 51 *Ae. sollicitans* were captured in the MMX trap. In the CDC light trap, a total of 499 *Ae. taeniorhynchus* and 149 *Ae. sollicitans*, with an average across all three replicates at 166 *Ae. taeniorhynchus* and 50 *Ae. sollicitans* were captured. The BG trap captured the lowest number of mosquitoes, with a total of 16 *Ae. taeniorhynchus* and 39 *Ae. sollicitans* and an average across all three replicates at five *Ae. taeniorhynchus* and 13 *Ae. sollicitans*. Although it seemed to perform well, the mosquito numbers captured by the MMX traps were not statistically significant compared to those captured by the CDC light traps and BG traps. However, there was a significant difference in abundance between *Ae. taeniorhynchus* and *Ae. sollicitans* in the MMX trap, though the difference is marginal ( $N = 6, \chi^2 = 3.8571, DF = 4, P = 0.0497$ ).

Despite the comparable salt marsh mosquito capture, efficacy between the three tested trap types, the MMX trap is preferred for capturing live mosquitoes suitable for the CDC bottle bioassay, due to its counter flow updraft system, which collects mosquitoes unharmed in the clear plastic capture chamber. Unlike the MMX traps, CDC light traps suck mosquitoes into capture jars using suction force generated through miniature fans. The mosquitoes contact the suction fan blades upon capture, which potentially dam-

Table 1. Average numbers of *Aedes taeniorhynchus* and *Ae. sollicitans* captured in the MMX trap, CDC light trap, and BG trap.

Species	MMX trap Average	CDC light traps Average	BG traps Average
<i>Aedes taeniorhynchus</i>	232.3 ± 83.5 <sup>Ba</sup>	166.3 ± 126.9 <sup>Aa</sup>	5.3 ± 3.5 <sup>Aa</sup>
<i>Ae. sollicitans</i>	51.3 ± 19.5 <sup>Aa</sup>	49.7 ± 27.1 <sup>Aa</sup>	13.0 ± 9.1 <sup>Aa</sup>

N = 3 traps.

Lowercase superscript letters indicate no significant difference for total species between trap types.

Uppercase superscript letters indicate a significant difference in abundance between the two species (P-value = 0.0497).

ages major appendages and thus confounds bottle bioassays. The CDC bottle bioassay detects mosquito susceptibility levels or resistance to active ingredients in insecticides by measuring their morbidity and mortality over designated exposure time (CDC, 2010). Damage to mosquitoes before testing can have a significant impact on the rate of mortality in control and treatment groups. For this reason, minimizing damage to mosquitoes during trap capture is a major consideration when collecting them from the field.

Although the mortality of mosquitoes caught in CDC light traps and MMX traps was not analyzed, future experiments could look at how the two suction systems affect downstream applications from field collected specimens. Future experiments could also test alternative attractants and new trap types for improved capture rates of *Ae. taeniorhynchus* and *Ae. sollicitans*. This study, ultimately, analyzed the capacity of different traps for capturing a high numbers of live undamaged salt marsh mosquitoes not only for insecticide susceptibility /resistance bioassays, but also for lab colony development, molecular testing, and other experiments that increase our understanding biological and ecology of the collected mosquito species for informed and targeted control operations.

#### ACKNOWLEDGEMENTS

We would like to thank Mr. Jim Cilek and James Richardson from the USDA/ CMAVE for their help with statistical analyses for this study.

#### REFERENCES CITED

- Hribar LJ, DeMay DJ, Lund UJ. 2010. "The association between meteorological variables and the abundance of *Aedes taeniorhynchus* in the Florida Keys." *Journal of Vector Ecology* 35 :339-346.
- Kline DL, Lemire, GF. 1995. Field evaluation of heat as an added attractant to traps baited with carbon dioxide and octenol for *Aedes taeniorhynchus*. *Journal of the American Mosquito Control Association-Mosquito News* 11:454-456.
- Nayar JK. 1985. Bionomics and physiology of *Aedes taeniorhynchus* and *Aedes sollicitans*, the salt marsh mosquitoes of Florida: University of Florida.
- Peng ZK, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FER. 2004. Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites. *Journal of Allergy and Clinical Immunology* 114 :1189-1194.
- Centers for Disease Control and Prevention (CDC), 2010. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. Atlanta: The Centers.
- Rey JR, Carlson DB, Brockmeyer RE. 2012. Coastal wetland management in Florida: environmental concerns and human health. *Wetlands Ecology and Management* 20:197-211. doi: 10.1007/s11273-011-9235-1.
- Rueda LM, Harrison BA, Brown JS, Whitt PB, Harrison RL, Gardner RC. 2001. Evaluation of 1-octen-3-ol, carbon dioxide, and light as attractants for mosquitoes associated with two distinct habitats in North Carolina. *Journal of the American Mosquito Control Association* 17:61-66.
- Smith KL, Qualls WA, Xue RD. 2016. Evaluation of talent UV light traps compared with CDC light traps with or without dry ice to collect fresh and salt water mosquitoes in Northeastern Florida. *TBFMCA* 10:91-92.
- Sudia WD, Newhouse VF, Henderson BE. 1971. Experimental infection of horses with three strains of Venezuelan equine encephalomyelitis virus II. Experimental vector studies. *American journal of epidemiology* 93:206-211.
- Weaver SC, Salas R, Rico-Hesse R, Ludwig GV, Oberste, Boshell J, Tesh RB VEE Study Group. 1996. Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America." *The Lancet* 348 (9025):436-440.

# COMPARATIVE EFFICACY OF FIVE PERMETHRIN/ PBO 30-30 GROUND ULV INSECTICIDES AGAINST FIELD COLLECTED ADULT *Aedes aegypti*, *Aedes* *taeniorhynchus*, AND *Culex quinquefasciatus* IN MANATEE COUNTY, FLORIDA

KATIE F. WILLIAMS<sup>1</sup>, EVA A. BUCKNER<sup>2</sup>, AMBYR L. MARICANO<sup>1</sup>,  
MARK D. LATHAM<sup>1</sup>, AND CHRISTOPHER R. LESSER<sup>1</sup>

<sup>1</sup>Manatee County Mosquito Control District, 2317 2nd Ave W, Palmetto, FL 34221

<sup>2</sup>University of Florida/IFAS, Florida Medical Entomology Laboratory,  
200 9th Street SE, Vero Beach, FL 32962

Subject Editor: Seth Britch

## ABSTRACT

We investigated five formulations containing synergized permethrin/PBO active ingredients, Biomist® 30-30, Evoluer® 30-30, Kontrol™ 30-30, Permanone® 30-30, and Perm-X™ UL 30-30, to determine whether there was variation in efficacy against caged local field collected adult *Aedes aegypti*, *Aedes taeniorhynchus*, and *Culex quinquefasciatus* mosquitoes. Mortality data from field trials with these formulations applied via truck mounted ultra-low volume sprays at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) label rates indicated generally low efficacy against *Ae. aegypti* and *Cx. quinquefasciatus* but generally high efficacy against *Ae. taeniorhynchus*. We discuss potential underlying mechanisms for this variation including effects of meteorology and resistance, and how field-derived efficacy data may be used operationally by mosquito and vector control districts to mitigate cost, environmental impact, and pesticide resistance.

Key Words: Adulticide, pyrethroid, permethrin, resistance

Chemical control with ground ultra-low volume (ULV) adulticides is an effective component of integrated vector management (IVM) to reduce arbovirus vector and nuisance biting mosquitoes (Faraji et al. 2016). Permethrin, a pyrethroid, is an active ingredient in adulticides commonly used in mosquito control due to its relative stability, high toxicity to a wide range of insects at low dosages, and rapid knock-down effects (Smith and Stratton 1986). Field trials with caged sentinel mosquitoes provide data on the potential efficacy of adulticide formulations against natural mosquito populations and, with increased insecticide resistance and environmental concerns, evaluations of different formulations are a necessary component of improved operational planning and decision-making (Farajollahi and Williams 2013). For example, in previous field trials we observed relatively low efficacy of

a formulation containing synergized permethrin and PBO against *Aedes aegypti* compared to formulations containing deltamethrin or plant-derived pyrethrins (Buckner et al. 2016). In that study, however, only one permethrin formulation was investigated. Thus, in the present study we investigated whether there is variation in efficacy among five ground ULV adulticide formulations each containing a 30-30 ratio of synergized permethrin and PBO active ingredients against field collected natural populations of adult *Aedes aegypti* (L.) as well as *Aedes taeniorhynchus* (Wiedemann), and *Culex quinquefasciatus* Say mosquitoes.

We conducted truck-mounted ULV spray trials over an open field targeting sentinel mosquito bioassay cages in an unfinished neighborhood, Sanctuary Cove, located in Palmetto, FL (27.516963 N, -82.544865 W) between June 14 and September 27, 2016.

Williams et al.: Efficacy of five permethrin

We investigated five formulations, Biomist® 30+30 (Clarke®, St. Charles, IL), Evoluer® 30-30 (AllPro®, Northville, MI), Kontrol™ 30-30 (MasterLine®, Austin, TX), Perma-none® 30-30 (Bayer, Research Triangle Park, NC), and Perm-X™ UL 30-30 (Central Life Sciences®, Schaumburg, IL), at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) label rates. For each formulation at each of the two application rates we performed at least two trial replicates. We performed a third replicate for Evoluer and Perm-X at the maximum label rate; however, weather impeded our ability to conduct third replicates for the remaining three formulations.

We conducted field cage bioassays for spray trials with field collected mosquitoes using methods described in WHO (2009). We distributed 9 sentinel cage poles for each trial across a 3 × 3 grid with 30.5 m (100 ft) separation between each pole in each row, with 3 rows of poles at 30.5 m (100 ft), 60.9 m (200 ft) and 91.4m (300 ft) downwind and perpendicular to the spray path. Additionally, we positioned two control sentinel cage poles and a weather station upwind of the spray area. We mounted treatment and control mosquito bioassay cages (8.5-cm diam., 14-cm height; see Buckner et al. 2016) above the level of the vegetation at 1.5 m on poles, with 3 separate bioassay cages containing 2030 female field collected *Ae. aegypti*, *Ae. taeniorhynchus*, or *Cx. quinquefasciatus* mosquitoes positioned on each pole for each trial. We paired each treatment and control bioassay cage pole with a rotating impinger based on the Florida Latham-Bonds design holding two 3 mm Teflon-coated acrylic slides to collect spray droplets.

The upwind weather station array consisted of a Kestrel® 4500 NV Model Pocket Weather® Tracker (KestrelMeters.com, Boothwyn, PA) recording temperature, wind direction, wind speed, and relative humidity at 5 sec intervals at ground level, and two DirecTemp® (QTI Sensing Solutions, Boise, ID) temperature probes on a PVC mast at 1.2 m and 10 m above ground and two NM100 Weather Stations (New Mountain Innovations, Inc., Old Lyme, CT) at 1.7 m and 10 m above ground to record wind speed and direction. We

used data streams from the weather station array to identify the presence of thermal inversions and whether temperature at both heights would be suitable for keeping the ULV spray at ground-level.

Starting in late May 2016, and throughout the summer we collected mosquitoes as eggs or larvae throughout Manatee County, FL and maintained them at Manatee County Mosquito Control District (MCMCD) under standard laboratory conditions at  $28 \pm 2$  °C and  $75 \pm 5\%$  RH, and photoperiod of 14:10

(L:D) h. We collected *Ae. aegypti* eggs from little black jars left outdoors in Cortez, FL, (27.459835 N, 82.664212 N) containing a piece of germination paper lining the rim of each jar, approximately 300 mL of water, and 5 g of a 3:2 mixture of liver powder and brewer's yeast. *Aedes taeniorhynchus* larvae were collected by MCMCD inspectors throughout the county, and we collected *Cx. quinquefasciatus* rafts from a Manatee County Wastewater Plant (27.488016 N, 82.373168 W). We transferred 2 to 10 day-old adult female mosquitoes reared from these eggs or larvae into bioassay cages using a mechanical aspirator (Hausherr's Machine Works, Toms River, NJ) approximately 5-6 h prior to testing and provided mosquitoes access to a cotton ball soaked with 10% sucrose solution. We stored cages in a designated large plastic tote with a lid in the insectary until transportation of the tote to the test site.

Once weather conditions were conducive to spraying at the test site, we activated the rotating impingers and hung mosquito bioassay cages on the poles 5 to 10 minutes prior to each spray trial. We used a truck mounted 18-20 London Fogger (Adapco® Inc, Sanford, FL) ULV aerosol generator for all spray trials driven at 4.4-8.94 m/s (10-20 mph) depending on the designated application rate, with a flow rate of 192 mL/min (6.5 fl oz/ min) monitored using a GeoTracker™ (Adapco Inc.). We collected all slides and cages 10 min post-application and immediately transported them back to the MCMCD laboratory. We exposed all bioassay mosquito cages to CO<sub>2</sub> for 28 sec to knock down and transfer mosquitoes to paperboard holding con-

tainers covered with mesh netting, supplied with cotton balls soaked with 10% sucrose solution, and kept at room temperature. We assessed mosquitoes for knockdown at 1 h and mortality at approximately 12 h post-application. After all trials were conducted, the mortality rates caused by the five permethrin/PBO 30-30 ULV products at mid and maximum application rates for each mosquito species were pooled to provide a mean. We calibrated all equipment/formulation combinations prior to the trials to produce a DV<sub>0.5</sub> or volume mean diameter (VMD), of 15-20 microns as measured using the waved 1-in slide method (Table 1). Additionally, we determined droplet density and DV<sub>0.5</sub> at each sampling station using DropVision® (Leading Edge Associates, Waynesville, NC) as soon as possible the same morning following each spray trial. Ground ULV trials were only conducted when wind velocity was 0.45-14.4 m/s (1-10 mph) and a temperature inversion existed. Within each of the seven test periods, ambient temperature ranged from 24.5 to 29.3 °C and local ground-level winds ranged from 0.94 to 3 m/s (2.1 to 6.7 mph).

During mid- label rate application trials, Permanone had the smallest mean droplet size (9.03 µm) and a mean droplet density of 26.81 drops/mm<sup>2</sup>, whereas Biomist had the largest mean droplet size (11.52 µm) and a mean droplet density of 43.36 drops/mm<sup>2</sup> (Table 1). During maximum label rate application trials, Permanone had the smallest mean droplet size (8.94 µm) and a mean droplet density of 22.50 drops/mm<sup>2</sup>, whereas Biomist had the largest mean droplet size (11.72 µm) and a droplet density of 54.84 drops/mm<sup>2</sup> (Table 1). These findings confirm that truck-mounted ULV applications for all five tested formulations produced droplets of optimal size and concentration meeting label specifications.

It is important to note that droplets were larger on the pre-trial 1-in calibration slides (Table 1). The apparently smaller droplet sizes measured during spray trials is expected and can be explained by two factors: 1) When sampling at a further distance from the nozzle, i.e., 30-90 m during spray trials compared to a few meters during calibra-

Table 1. Mean droplet size ±SE and mean droplet density ±SE for five ground ULV oil-based 30% permethrin 30% PBO adjuvanted formulations applied at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) application rates.

Treatment	Biomist ml/ha 113 226	Evolver ml/ha 113 226	Kontrol ml/ha 113 226	Permanone ml/ha 113 226	Perm-X ml/ha 113 226
DV <sub>0.50</sub> (µm; VMD) <sup>1</sup>	11.72 ± 0.65    11.52 ± 0.38	10.11 ± 0.60    11.10 ± 0.35	9.43 ± 0.25    9.56 ± 0.40	8.94 ± 0.95    9.03 ± 1.00	10.76 ± 0.29    10.71
Droplet Density (mm <sup>2</sup> )	54.84 ± 20.74    43.36 ± 8.11	29.68 ± 0.84    35.28 ± 3.27	45.55 ± 2.71    20.45 ± 3.22	22.50 ± 1.09    26.81 ± 5.25	40.78 ± 2.20    4.14
Wave Slide DV <sub>0.50</sub> (µm; VMD)	17.91 ± 2.31    16.83 ± 1.22	18.30 ± 0.69    17.20 ± 0.42	17.22 ± 3.65    15.26 ± 1.68	17.51 ± 0.08    17.51 ± 0.08	18.43 ± 0.14    18.29

<sup>1</sup>VMD, volume mean diameter

Williams et al.: Efficacy of five permethrin

tion, larger droplets should settle out prior to reaching the spinning slides, and 2) the narrow (3 mm) slides used during spray trials to better sample the diffuse spray cloud (Bonds et al. 2009) have a biased collection efficiency for small droplets compared to a 1-in slide, causing underestimation of true droplet sizes (Fritz et al. 2011).

Mortality for both *Ae. aegypti* and *Cx. quinquefasciatus* was relatively low for all five formulations (Fig. 1 A and C), not exceeding approximately 67% (mid application rate) and 72% (maximum rate). On the other hand, mortality for *Ae. taeniorhynchus* was relatively high for all five formulations (Fig. 1 B), with up to approximately 96% (mid rate) and 100% (maximum rate). Comparing 1 h knockdown to 12 h mortality, we observed 4% increased mortality in *Ae. aegypti* and 0.66% in *Ae. taeniorhynchus*. In contrast, *Cx. quinquefasciatus* showed a 13% recovery between the knockdown and final mortality counts. Control mortality was <1% during this experiment.

Mosquito control districts including MC-MCD leverage ground ULV applications of formulations containing synergized permethrin as a key IVM component to prevent arbovirus transmission and reduce mosquito populations before they become a nuisance (Faraji et al. 2016). It is important that these applications are efficacious while using as little insecticide as possible (Mount 1998), and droplets must drift through areas where mosquitoes are flying or resting to be effective. In this investigation we consistently collected droplets throughout the treatment grid with droplet sizes that met label requirements for both mid- and maximum label rates, so the observed mortalities in the field collected sentinels should be a good indication of the potential relative efficacies of these formulations in an operational setting using similar calibrated pieces of equipment.

Out of all five formulations tested, Kontrol performed the best against all three species when applied at the maximum label rate, resulting in mortality rates of 70%, 100%, and 72% for *Ae. aegypti*, *Ae. taeniorhynchus*, and *Cx. quinquefasciatus*, respectively. If

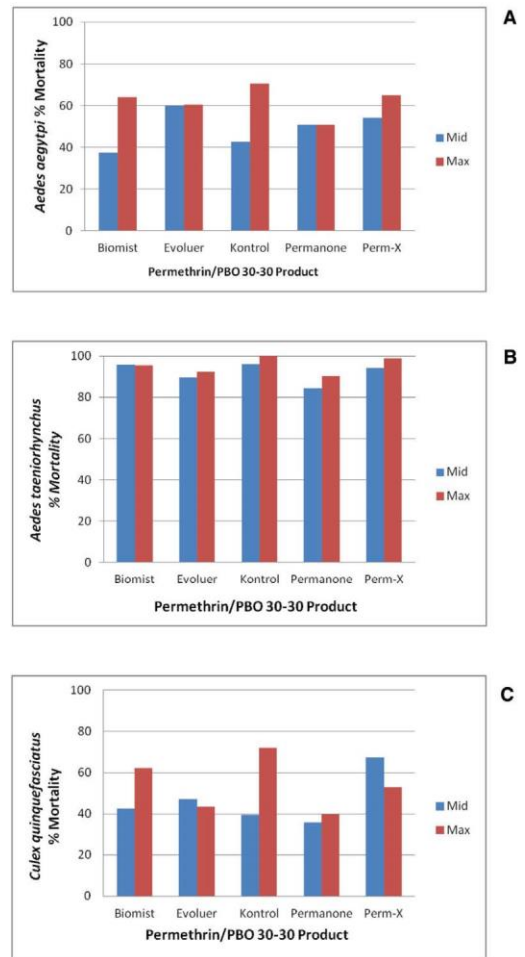


Figure 1. Mean mortality 12 h post-application for caged (A) *Ae. aegypti*, (B) *Ae. taeniorhynchus*, and (C) *Cx. quinquefasciatus* mosquitoes exposed to five ground ULV oil-based 30% permethrin + 30% PBO adulticide formulations applied at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) application rates.

we only consider targeting *Ae. taeniorhynchus* using a permethrin/PBO 30-30 product, our results indicate that four of the formulations, Biomist, Evoluer, Kontrol, and Perm-X, achieved mortality rates  $\geq 90\%$  when applied at mid-rate, while Permanone only met that benchmark at the maximum rate important considerations in cost comparisons across formulations balanced with minimizing introduction of pesticides into the environment.

Biomist, Evoluer, Perm-X, and Permanone resulted in mortality <70% against *Ae. aegypti* and *Cx. quinquefasciatus*, independent



of application rate. This could have been due to permethrin resistance developing in local *Ae. aegypti* and *Cx. quinquefasciatus* populations (Kasai et al. 2014) that may be more pronounced with certain formulations of permethrin and PBO active ingredients. Future studies should test natural populations of these species in Manatee County to evaluate permethrin resistance levels across these formulations. Aside from resistance, unfavorable weather conditions could have decreased efficacy in some trials. Wind direction and velocity, as well as temperature play important roles in successful ground ULV applications (Cornine 2015). During application for each trial, wind velocity was 1-10 mph at ground level and we confirmed the presence of a thermal inversion that should have trapped droplets near the ground and the sentinel cages as they drifted through the treatment area (Mount 1998). However, in the field weather can change rather rapidly and if the wind shifts or temperature changes droplets may not reach all of the cages in the treatment grid. This may have contributed to the results we saw when spraying Perm-X against *Cx. quinquefasciatus*, where we observed increased control (67.52%) at the mid rate compared to 52.86% at maximum rate. Also, the majority of these products were only tested at each application rate twice instead of three times due to unfavorable weather conditions, and additional replicates could have revealed different patterns of efficacy.

Overall, the results of this investigation provide evidence that ground ULV applications of formulations containing permethrin/PBO 30-30 active ingredients may be effective against local populations of mosquitoes in Manatee County, with variation depending on target species and application rate. Looking forward, MCMCD should continually monitor the efficacy of these and other adulticide formulations used in their IVM program. Effective IVM programs should incorporate efficacy data such as pre-

sented here along with resistance testing and cost analyses to design seasonal rotations of active ingredients to mitigate resistance.

A special thanks goes out to the dedicated staff at MCMCD, including D. Andress, C. Bustle, P. Conrad, J. Davis, W. Thompson, J. Jackson, and M. Geesey for all of their assistance during these research trials.

## REFERENCES CITED

- Buckner E, Latham M, Lesser C, Marsicano A, K Williams. 2016. An evaluation of 6 ground ULV adulticides against local populations of *Aedes taeniorhynchus* and *Aedes aegypti* in Manatee County, FL. *Wing Beats* 27: 17-19.
- Bonds J, Greer M, Fritz B, Hoffman W. 2009. Aerosol sampling: comparison of two rotating impactors for field droplet sizing and volumetric measurements. *J Am Mosq Control Assoc.* 25 (4): 474-479.
- Cornine F. 2015. Efficacy trials of the Central Massachusetts mosquito control project residential adulticide program. Update 2015. Massachusetts Mosquito Control Project, Northborough, MA [accessed November 15, 2017]. Available from: [http://www.cmmcp.org/sites/cmmcp/files/uploads/2015\\_efficacy\\_trials\\_of\\_mosquito\\_control\\_adulticide\\_program.pdf](http://www.cmmcp.org/sites/cmmcp/files/uploads/2015_efficacy_trials_of_mosquito_control_adulticide_program.pdf)
- Faraji A, Unlul I, Crepeau T, Healy S, Crans S, Lizarraga G, Fonseca D, Gaugler R. 2016. Droplet characterization and penetration of an ultra-low volume mosquito adulticide spray targeting the Asian Tiger Mosquito, *Aedes albopictus*, with urban and suburban environments of northeastern USA. *PLoS ONE.* 11 (4): e0152069.
- Farajollahi A, Williams G. 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.
- Fritz B, Hoffman W, Bonds J, Farooq M. 2011. Volumetric collection efficiency and droplet sizing accuracy of rotary impactors. *Am Soc of Ag and Bio Eng.* 54(1): 57-63.
- Kasai S, Komagata O, Itokawa K, Shono T, Ng LC, Kobayashi M, Tomita T. 2014. Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: Target site insensitivity, penetration, and metabolism. *PLOS Negl Trop Dis* 8(6): e2948.
- Mount, GA. 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.
- Smith TM, Stratton GW. 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. In: Gunther FA (eds) *Residue Reviews*. Residue Reviews, vol 97. Springer, New York, NY.
- WHO [World Health Organization]. 2009. *Guidelines for efficacy testing of insecticides for indoor and outdoor ground-applied space spray applications*. Document WHO/HTM/NTD/WHOPES/2009.2. Geneva Switzerland: World Health Organization.

# EVALUATION OF A NEW TRUCK-MOUNTED ULV SPRAYING MACHINE WITH *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST LARVAL *CULEX QUINQUEFASCIATUS*

LEI LUO<sup>1</sup>, MARCIA K. GAINES, AND RUI-DE XUE

<sup>1</sup>Anastasia Mosquito Control District, 120 EOC Drive,  
St. Augustine, FL, USA 32092, USA

Guangzhou City's Centre for Disease Control and Prevention,  
Guangzhou, China

Guest Editor: M. Farooq

## ABSTRACT

A field study about the effectiveness of a new truck-mounted ultra-low-volume (ULV) machine against larval *Culex quinquefasciatus* Say was conducted at Anastasia Mosquito Control District of St. Johns County, St. Augustine, FL, during the summer of 2017. *Cx. quinquefasciatus* larvae were treated using a ground application at different concentrations of Bti using a new truck-mounted ULV sprayer with a horizontal nozzle. Mortality of larvae was recorded after 24 h, and droplet sizes were measured. Overall, Bti sprayed by the new truck-mounted ULV spraying machine at a concentration of 2.625 mg/L resulted in the highest mortality of mosquito larvae. The results indicate that the Bti concentrations of 0.875 mg/L and 0.065 mg/L resulted in a significant difference in mean larval mortality between each distance from the spray line ( $P < 0.05$ ), while the mortality by the other 3 concentrations (0.477 mg/L, 2.625 mg/L, and 5.25 mg/L) tested did not. The LC<sub>50</sub> and LC<sub>90</sub> of Bti against larvae were 0.261 mg/L (0.239~0.286) and 1.687 mg/L (1.481~1.922), respectively. The coverage (swath) of the spray by the new ULV machine showed that the Bti could be sprayed at least 33 meters away with a 20 meter width. Therefore, the new truck-mounted ULV spraying machine with liquid Bti could be used to treat a large area effectively and efficiently and as an additional tool for the control of mosquito larvae.

Key Words: *Culex quinquefasciatus*, larvae, mortality, *Bacillus thuringiensis israelensis*, truck-mounted ULV spraying machine

## INTRODUCTION

Mosquitoes are a nuisance and many play potential vectors of human diseases, such as malaria, filariasis, and arboviral diseases (dengue fever, Chikungunya, West Nile fever, Zika, and yellow fever) (Mittal 2003). Integrated vector management (IVM), targeting both larval and adult mosquitoes, has been considered the most effective measure for mosquito elimination (Imbahale et al. 2012). Vector control efforts typically target the immature stages and controlling their population is an important task for public health (Lucia et al. 2009). Applying larvicides in breeding sites is still considered a priority for mosquito control (Williams et al. 2014). The traditional approaches to lar-

val control are to find the larval sources and empty/treat those using hand-held sprayers or truck-mounted sprayers with different formulations of larvicides. Previous research illustrated the effectiveness of a hand-pump and gasoline powered backpack sprayer (Sandoski et al. 1985), truck-mounted cold aerosol fogger, Buffalo Turbine mist sprayer (Williams et al. 2014), ULV (Lam et al, 2010, Harwood et al. 2016), and even aerial spraying (Pruszynski et al. 2017) for the control of mosquito larvae.

*Bacillus thuringiensis israelensis* (Bti), a bacterial larvicide which is highly target specific and appropriate for wide area spraying, is used to control mosquito and black fly larvae (Yap et al. 1997). Different formulations of Bti have been successfully used in

ground applications with hand-pump backpack sprayers/misters, and thermal foggers (Dunford et al. 2014). These applications have a limited utility for large scale control. Truck-mounted cold aerosol foggers have been studied for the large scale control of mosquito larvae over time (Williams et al. 2014). However, their utility has still to be appreciated. Thus, continuous search and evaluation of new effective and economic spray machines for efficient control should concern professionals.

The new truck-mounted ULV spray machine used in this project has been evaluated for barrier treatments (Fulcher et al 2015) but has not been tested for the application of any kind of larvicides. The objectives of this study were to 1) determine whether the new truck-mounted ULV spray machine can be used to apply liquid Bti to control larval *Culex quinquefasciatus* Say in artificial containers, 2) determine the optimal dilution of Bti for larval control by using the new machine, and 3) measure the distance and width (swath) reached by the new machine.

## MATERIALS AND METHODS

**Study site and mosquito larvae.** This study was conducted at the Anastasia Mosquito Control District (AMCD), 120 EOC Drive, St. Augustine, FL, on an unpaved and grassy ground. A susceptible strain of *Cx. quinquefasciatus* Say larvae was provided by the AMCD insectary. Late third- or early fourth-instar larvae were used for the study.

**Insecticide.** A commercial biolarvicide named AQUABAC®xt (a.i. *Bacillus thuringiensis* var. *israelensis* (Bti), Becker Microbial Products, Inc. Plantation, FL), which contained 8% *Bacillus thuringiensis* subspecies *israelensis* Strain BMP 144 solids, spores and insecticidal toxins, was used for the experiment. The formulation of the biolarvicide is an aqueous suspension. The label application instructions specify the rate of AQUABAC®xt required for control with a sufficient amount of water to provide thorough coverage of the target area when it is applied in conventional aerial and ground application equipment. The formulation was

diluted in purified water at 1:0, 1:1, 1:5, 1:10 and 1:80 (Bti:Water) ratios to provide active ingredient concentrations of 5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L, respectively.

**Equipment.** The new truck-mounted ULV spray machine (model: LR-4P, 4 nozzles installed on 4 spray heads, American LongRay, Inc., San Francisco, CA) was evaluated in this study. The machine has excellent atomization, quick diffusion, and creates a dense fog that penetrates into spaces and lingers in the air for a longer time. Also, the spray machine heads can be adjusted to swivel 360 degrees horizontally and 180 degrees vertically by manual or remote control. This Truck-mounted cold fogger can spray a variety of chemicals used for public health protection, disinfection, vector control, pest control, and crop protection. This equipment produced droplets at a volume median diameter (Dv0.5) of 14.3 µm and 90th percentile (Dv0.9) of 30.4 µm while spraying water.

**Field test.** Between 20-30 mosquito larvae were collected using a plastic aspirator from a stock plastic pan (50 cm L × 38 cm W × 7 cm H) and transferred to each of nine white plastic containers (30 cm L × 18 cm W × 10 cm H) with 2 L purified water. The dilutions were sprayed with the new truck-mounted ULV spray machine at a flow rate of 92 ml/min. At a travel speed of 16 Km/h, the certified pesticide sprayer applied the different Bti dilutions at an application rate of 37 ml/ha.

For the optimal concentration in each treatment, all four nozzle heads were angled horizontally (parallel to the ground). The effectiveness of each application was assessed by the mortality of *Cx. quinquefasciatus* larvae at 24 h post treatment.

Three rows of larval containers (from No 1 to No 9) at 2 m apart were placed up to 15 m perpendicular to the spray line. In each row, 3 containers were separated at 5, 10, and 15 m away from the spray line. Alongside treatment container No. 3, 7, and 9, a Florida Latham Bond spinner (model 319; John W. Hock Company, Gainesville, FL) using a 3 mm × 75 mm Teflon® coated slide was deployed to determine spray droplet

sizes. Larval containers were placed on the ground and the spinners were hung at 1.2 m height on the posts. Spray application of each concentration was replicated three times at the same site, with at least one week interval between trials. For each replication, three control larval containers were deployed for 15 minutes at the first row of the spray zone (5 m from the spray line) and were removed before start of the spray. Test larval containers and spinner slides were placed in the field immediately before spray. The truck-mounted ULV sprayer was operated at a speed of 16 Km/h from 17 m before the test area and ended 17 m past the test area. Spinner slides and test larval containers were removed from the field at 5 min and 15 min, respectively post-treatment. The air temperature (25-28 °C), relative humidity (65-90%), wind speed (3-15 Km/hr), and wind direction were recorded at 1.5 m above ground during the field tests. After applications, all larval containers were transferred to the AMCD laboratory and larval mortality counts were taken at 24 h post treatment. All slides were transported to the laboratory for droplet size measurements. Droplets on the slides were measured with DropVision system (Leading Edge, Fletcher, NC) and droplets statistics were determined with the associated software.

The distance and width (swath) reached by the new truck-mounted ULV sprayer was tested using circular plastic disks set in an array at fixed distances. The machine's tank was filled with a mixture of water and red dye to visually detect the swath on the disks. Four rows of collectors, 7 m apart, were set up to 33 m perpendicular to the sprayer on either direction. In each row, four, 4.72 cm diameter circular plastic disks, each with a piece of white test filter paper, were held on the ground at 13, 20, 27, and 33 m from the sprayer. The sprayer was oriented towards the test array and was stationed in the center of the array. Two tests were conducted. The first test was for all four nozzle heads sprayed toward the same direction horizontally (parallel to the ground). The second test divided the four nozzles into two groups, one set spraying horizontally (parallel to the

ground) to one direction and the other oriented toward the opposite direction. Each test spray lasted for 10 seconds. All the white test filter papers were collected and examined under the microscope for the red dye to assess the distance and width (swath) which the sprayer can reach.

**Date analysis.** The larval mortality was corrected by Abbott's formula (Abbott 1925) when a 5% or more mortality was detected in the control group. The values for larval mortality of each replicate in each test were calculated and the dose-mortality response was assessed using the R-script BioRssay package, which computes the doses of insecticides killing 50% (LC50) and 90% (LC90) of the tested larvae. For the comparison of 24 hour mortality between the 5 different concentrations (5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L) and 3 distances (5 m, 10 m, and 15 m) from the spray line with droplet Dv0.5, nonparametric Kruskal-Wallis analysis and multifactor analysis of ANOVA were conducted, respectively. A posteriori of Tukey HSD means multiple comparison tests was conducted by using Software STATGRAPHICS Plus for Windows Version 4.0-CorpsGWIN P®. Analysis was conducted with R software (Version 3.4.1). A p-value of <0.05 was considered statistically significant.

## RESULTS

The Bti dilution ratio by water (1:0, 1:1, 1:5, 1:10, and 1:80) was converted to the actual active ingredient concentrations of 5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L, respectively. There was a significant difference in 24 h larval mortality between each test and control at all test concentrations ( $P < 0.05$ ) (Fig. 1).

As shown in Table 1, there was a significant difference in 24 hr mortality between different Bti concentrations ( $\chi^2 = 77.98$ ,  $P < 0.001$ ). The concentration of 2.625 (1:1) mg/L resulted in the highest larval mortality (100.00%), while 0.065 mg/L resulted in the lowest larval mortality (24.26%). Tukey multiple comparison tests identified that the mortalities from different test concentrations were significantly

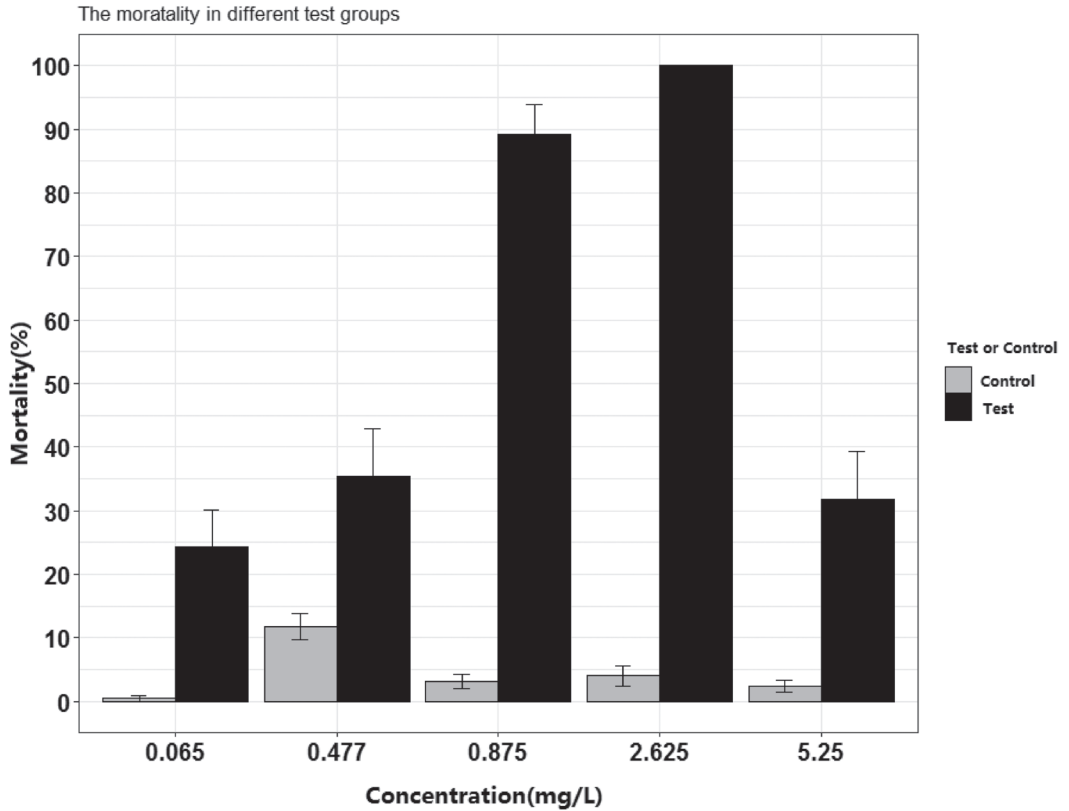


Fig. 1. Mean mortality (% ±SD) of larvae caused by Bti at different concentrations sprayed by the new truck-mounted ULV spraying machine, compared with mortality in control group

different from each other. The mortality from 0.875 mg/L and 2.625 mg/L was generally similar and were significantly higher than those of the other three concentrations tested (0.065 mg/L < 5.250 mg/L < 0.477 mg/L). The LC50 and LC90 of the larvae for Bti were 0.261 mg/L (0.239~0.286 mg/L) and 1.687 mg/L (1.481~1.922 mg/L), respectively, computed by the equation  $Y = 1.658 + 2.713 * X$  which was assessed by the dose-mortality response regression analysis.

The comparison of mean larval mortality between distances from the spray line from

each concentration (Table 2) indicated that distance significantly affected mortality from the concentrations of 5.250 mg/L and 0.875 mg/L ( $P < 0.05$ ). However, from the other 3 concentrations (0.477 mg/L, 2.625 mg/L, and 0.065 mg/L) distance did not significantly affect mortality. The concentrations of 0.875 mg/L and 2.625 mg/L at all distances produced 100% larval mortality except for the 15 m distance of 0.875 mg/L concentration, which produced 67.43% larval mortality only. Other concentrations at

Table 1. Mean larval mortality at 24 hr exposure to different concentrations of Bti sprayed by the new truck-mounted ULV spraying machine.

Concentration	N	Mean mortality%	SD	SE	95%CI	K-W( $\chi^2$ value)	P
0.065 mg/L	27	24.26	30.15	5.80	12.33~36.19	77.979	0.000
0.477 mg/L	27	35.44	38.36	7.38	20.26~50.62		
0.875 mg/L	27	89.14	24.73	4.76	79.36~98.92		
2.625 mg/L	27	100	0.00	0.00	100~100		
5.250 mg/L	27	31.85	38.55	7.42	16.6~47.1		

Table 2. Comparison of larval 24h mortality at different distances from the spray line after exposure to different concentrations of Bti sprayed by the new truck-mounted ULV sprayer.

Concentration	Distance from spray line	N	Mean mortality	SD	SE	CI	K-W( $\chi^2$ value)	P
0.065 mg/L	5m	9	39.34	31.08	10.36	15.45-63.23	4.970	0.083
	10m	9	10.91	13.71	4.57	0.37-21.45		
	15m	9	22.52	36.63	12.21	0-50.68		
0.477 mg/L	5m	9	39.91	38.63	12.88	10.21-69.61	0.088	0.916
	10m	9	33.92	41.20	13.73	2.25-65.59		
	15m	9	32.51	39.47	13.16	2.17-62.85		
0.875 mg/L	5m	9	100	0.00	0.00	100-100	11.661	0.003
	10m	9	100	0.00	0.00	100-100		
	15m	9	67.43	34.52	11.51	40.9-93.96		
2.625 mg/L	5m	9	100	0.00	0.00	100-100	—	—
	10m	9	100	0.00	0.00	100-100		
	15m	9	100	0.00	0.00	100-100		
5.250 mg/L	5m	9	44.62	44.21	14.74	10.63-78.61	0.751	0.482
	10m	9	23.44	36.61	12.2	0-51.58		
	15m	9	27.49	35.37	11.79	0.30-54.68		

all the distances produced similar mortality, which were lower than 50%.

There was a significant difference in droplet size Dv0.5 between different test concentrations ( $P < 0.05$ ). At the 0.065 mg/L concentration, the droplet size Dv0.5 was the biggest (31.49  $\mu\text{m}$ ), but the smallest (18.42  $\mu\text{m}$ ) was at the concentration of 2.625 mg/L (Table 3).

During the first spray test for distance and width (swath), all four nozzles sprayed in the same direction at the same time under SE 1 mph wind. After analysis using light microscopy, 10 out of 16 (62.50%) plates with white filter paper in this test had been stained with red dye. The number of disks with white filter paper stained in each row were 3, 2, 2 and 3 from 13, 20, 27, and 33 m away from the truck sprayer, respectively.

When four nozzles were divided into two groups (two in each group) and sprayed

towards two opposite direction under SE 2 mph wind, 11 out of 16 (68.75%) plates with white filter paper at the downwind side had been stained and the number of disks with white filter paper stained in each row were 4, 3, 3 and 1 from 13, 20, 27, and 33 m away from the truck sprayer, respectively. None of plates with white filter paper had been stained at the upwind side. These results indicate that spray reached up to 33 m in the downwind of the spray and did not travel much to the upwind side.

## DISCUSSION

When liquid is used to treat mosquito breeding sites, such as tidal water, salt marshes, catch basins, and storm water retention areas, we usually use conventional spraying equipment or apply our treatments directly in the breeding sites (Xue and Doyle 2005).

Table 3. Droplet size (Dv0.5) of the liquid Bti at different concentrations sprayed by the new truck-mounted ULV spraying machine.

concentration	N	Droplet size(Dv0.5)	SD	SE	CI	chi-square	P
5.250 mg/L	27	31.92	1.48	0.28	0.90~2.06	90.29	0.000
0.477 mg/L	27	24.72	2.77	0.53	1.67~3.87		
0.875 mg/L	27	22.58	4.01	0.77	2.42~5.60		
2.625 mg/L	27	18.42	4.82	0.93	2.91~6.73		
0.065 mg/L	27	31.49	4.00	0.77	2.42~5.58		

Our findings indicate that all 5 Bti concentrations (5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L), resulted in 24.26%-100% larval mortalities after 24 hours of exposure to liquid Bti (1,200 ITU/mg). The significant difference in larval mortality from all concentrations and control and > 90% mortality in some concentrations suggested that the new cold ULV spraying machine could be used to spray liquid Bti to kill mosquito larvae.

The new ULV spray machine with the highest and the lowest dilution ratios of Bti didn't result in significant mosquito larval mortality. The concentrations of 0.785 mg/L and 2.625 mg/L resulting from 1:5 and 1:1 dilutions, respectively were the suitable for the new machine to spray a large area.

The analysis of the larval mortality at different distances from the spray line suggested that the acceptable larvae mortality of >80% within a 15 m distance from spray could be obtained at the dilution ratio of 1:1 (2.625 mg/L) or up to 10 m with dilution ratio of 1:5 (0.875 mg/L).

Droplet size is a very important factor (Harburguer et al. 2012) for killing larvae using Bti (Seleena et al. 1996). In our study, we set three groups of teflon-coated slides atop spinners for droplet size analysis at different distances from the spray line to check the DV0.5. The droplet size data analysis and mortality relationship indicated that the smaller DV0.5 spray resulted in higher larval mortalities.

Additionally, the results of the distance and width reached by the new ULV spray suggests that the farthest distance (swath) should be no more than 33 m along wind when the new truck-mounted ULV spray machine is fixed at a central position spraying towards one side or two sides with nozzles oriented horizontally. Also, it was shown that the droplets could travel further and were more effective with all four nozzles sprayed towards same direction at the same time than that of subgroups (two nozzles for each group) sprayed towards two opposite direction at the same time. Also the distance and width can be dramatically affected by wind speed and direction from the two opposite

directions. But this test was conducted by using dyed water only, and the actual effective distance and width (swath) of the ULV spray machine needs to be addressed further. Finally, the study was conducted in a simulated field with a laboratory-reared sensitive strain of mosquitoes. A large-scale community field test needs to be carried out in the future.

The new truck-mounted ULV spray machine with liquid Bti provided efficient control for mosquito larvae in containers placed on the ground in the field trails. The new ULV spray machine may cover at minimum a 33 m distance x 20 m width. Bti sprayed with the new truck-mounted ULV spray machine is practical and could be considered as an alternative method for treatment for the large scale control of larval mosquitoes.

#### ACKNOWLEDGMENTS

The authors thank C. Bibbs for droplet analysis, D. Dixon for editing & reviewing the manuscript, C. Mangum for providing mosquito larvae, J. Wynn for calibration of the new machine, and J. Davis for participation in the experiment at the Anastasia Mosquito Control District, St. Augustine, FL.

#### REFERENCES CITED

- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265-267.
- Dunford JC, Stoops CA, Estep AS, Britch SC, Richardson AG, Walker TW, Farooq M, Platt RR, Smith VL, Wirtz RA, Kerce JD. 2014. SR450 and super hawk XP applications of *Bacillus thuringiensis israelensis* against *Culex quinquefasciatus*. *J Am Mosq Control Assoc* 30:191-198.
- Fulcher A, Farooq M, Smith ML, Li CX, Scott JM, Thomson E, Kaufman PE, Xue, RD. 2015. Evaluation of a new spraying machine for barrier treatment and penetration of bifenthrin on vegetation against mosquitoes. *J Am Mosq Control Assoc* 31:85-92.
- Harburguer L, Seccacini E, Licastro S, Zerba E, Masuh H. 2012. Droplet size and efficacy of an adulticidal-larvicide ultralow-volume formulation on *Aedes aegypti* using different solvents and spray application methods. *Pest Manag Sci* 68:137-141.
- Harwood JF, Helmey WL, Turnwall BB, Justice KD, Farooq M, Richardson AG. 2016. Controlling *Aedes aegypti* in cryptic environments with manually carried ultra-low volume and mist blower pesticide applications. *J Am Mosq Control Assoc* 32:217-223.
- Imbahale SS, Githeko A, Mukabana WR, Takken W. 2012. Integrated mosquito larval source management reduces larval numbers in two highland vil-

- lages in western Kenya. *BMC Public Health* 12, Article #362.
- Lam PH, Boon CS, Yng NY, Benjamin S. 2010. *Aedes albopictus* control with spray application of *Bacillus thuringiensis israelensis*, strain AM 65-52. *Southeast Asian J Trop Med Public Health* 41:1071–1081.
- Lucia A, Harburguer L, Licastro S, Zerba E, Masuh H. 2009. Efficacy of a new combined larvicide-adulticidal ultralow volume formulation against *Aedes aegypti* (Diptera: Culicidae), vector of dengue. *Parasitol Res* 104:1101-1107.
- Mittal P. 2003. Biolarvicides in vector control: challenges and prospects. *J Vect Borne Dis* 40:20–32.
- Pruszyński CA, Hribar LJ, Mickle R, Leal AL. 2017. A large scale biorational approach using *Bacillus thuringiensis israeliensis* (Strain AM65-52) for managing *Aedes aegypti* populations to prevent Dengue, Chikungunya and Zika transmission. *PLOS One* 12(2):e0170079. doi:10.1371/journal.pone.0170079.
- Sandoski CA, Yates MM, Olson JK, Meisch MV. 1985. Evaluation of Beecomist-applied *Bacillus thuringiensis* (H-14) against *Anopheles quadrimaculatus* larvae in rice field. *J Am Mosq Control Assoc* 1:316–319.
- Seleena P, Lee HL, Nazni WA, Rohani A, Kadri MS. 1996. Microdroplet application of mosquitocidal *Bacillus thuringiensis* using ultra-low-volume generator for the control of mosquitoes. *Southeast Asian J Trop Med Public Health* 27:628–632.
- Williams GM, Faraji A, Unlu I, Healy SP, Farooq M, Gaugler R, Hamilton G, Fonseca DM. 2014. Area-wide ground applications of *Bacillus thuringiensis var. israelensis* for control of *Aedes albopictus* in residential neighborhoods: from optimization to operation. *PLOS one*. 9(10):e110035. Doi10.1371/journal.pone.0110035.
- Xue RD, Doyle MA. 2005. Evaluation of upstream point treatment in flowing water ditches by aquabact (*Bacillus thuringiensis var. israelensis*) against *Culex quinquefasciatus*. *J Am Mosq Control Assoc* 21:234–235.
- Yap HH, Chong ASC, Adanan CR, Chong NL, Rohaizat B, Malik AY, Lim SY. 1997. Performance of ULV formulations (Pestguard® 102/Vectobac® 12AS) against three mosquito species. *J Am Mosq Control Assoc* 13:384–388.



# **SUBMITTED ABSTRACTS OF THE 90TH ANNUAL MEETING**

## **SMART PHONE APPLICATION FOR SUBMITTING PUBLIC SERVICE REQUESTS**

JAMES H. RICHARD WEAVER AND RUI-DE XUE

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Traditional methods of receiving a public service request was for a member of the public to call our mosquito control district and a member of our staff would write down the request and pass the request to the proper mosquito control technician, this could take several days. In 2010 the District developed a web-based service request program that allowed the public to enter their own service requests and the program automatically transferred those requests to the mosquito control technician in that area. This innovation reduced phone calls to the District from the public by 50%. Starting in the 2016's the District started receiving complaints that the web-based program was not able to be accessed by portable device such as smart phones and tablets. In the fall of 2017 the Board of Commissioners decided to develop a service request phone application (app). In the spring of 2018 the new phone app developed by GeoWorld Outdoors and Anastasia Mosquito Control District (AMCD) for Apple and Android devices was released to the public. The app allows the public to enter service requests, look up fogging maps, look up the status of a previously submitted service request, set up a permanent profile and receive push notifications. When the app is opened the home, page displays a map with the user's location, a drop-down menu allows the user to choose what they want to do. The service request entry field allows the user to enter the customer infor-

mation using the users profile or manually and entering the location information using the users profile, manually or using the maps (phones) current location. When the customer hits send the service request is sent directly to the mosquito technician allowing service to be preformed within minutes. The service request history menu item allows a customer to put in an address and date range and check to see the status of a service request previously submitted. Fogging maps can be viewed by entering an address and date range, if a fogging mission has been preformed for this address the treatment polygon will be displayed on the phones map. AMCD can also issue a push notification and notify any customers that have the app of important news, for example emergency aerial fogging or a health alert. With this new app AMCD has seen response time for service reduced to a median response time of .9 days, this is in spite of major increase in population and the number of service requests. In the first year of the app release we have seen 346 down loads of the app (265 iPhone, 81 Android) and now 73% of our service requests come via the web site or phone app. The publics reception to the phone app has been extremely positive and AMCD will continue to advertise the app using our education program. The app may also become a more important tool for both AMCD and the public as AMCD develops our new aerial program in the 2019 season.

## **ANALYSIS OF THE VOLTAGE-GATED SODIUM CHANNEL (VGSC) TARGET SITE MUTATION, L1014F, AND PYRETHROID RESISTANCE IN *CULEX QUINQUEFACIATUS* POPULATIONS OF COLLIER COUNTY, FL**

KEIRA J. LUCAS<sup>1\*</sup>, KACI MCOY<sup>1,2</sup>, CAROLINE WELDON<sup>1,2,3</sup>  
AND RACHEL B. BALES<sup>1</sup>

\*Presenter

<sup>1</sup>Collier Mosquito Control District, 600 North Road Naples, FL 341042

<sup>2</sup>School of Public Health and Tropical Medicine, Tulane University,  
1440 Canal Street New Orleans, LA 701123

<sup>3</sup>CDC Southeastern Center of Excellence in Vector Borne Disease,  
2055 Mowry Road, Gainesville FL 32611

In several insect species, resistance to pyrethroids and dichloro-diphenyl-trichloroethane (DDT) is linked to point mutations in the voltage-gated sodium channel (VGSC) gene. Pyrethroid-based insecticides prolong the opening of sodium channels, causing paralysis known as a “knockdown” effect. Point mutations in the VGSC gene results in decreased pyrethroid binding and reduced sensitivity to the insecticide – this resistance mechanism is known as knockdown resistance (*kdr*). In *Culex* mosquito species loss of target site sensitivity to pyrethroids is caused by a substitution of leucine (L) to phenylalanine (F) at residue 1014 (L1014F) in the VGSC gene. Here we report the identification of *kdr*-associated pyrethroid resistance

and developing resistance in *Cx. quinquefasciatus* field populations from Collier County, Florida (FL). Evaluation of position 1014 of the VGSC in *Cx. quinquefasciatus* populations from eight locations in Collier County FL revealed a wide range of genotype and allele frequencies from one part of the district to the other. CDC bottle bioassay, linear regression analysis and cage trial evaluations suggest that the L1014F mutation plays a role, at least in part, to the pyrethroid resistance status of *Cx. quinquefasciatus* collected in Collier County FL. Furthermore, we suggest that the frequency of L1014F allele can serve as an indicator of pyrethroid resistance in field populations of *Cx. quinquefasciatus*.

## **REAL-TIME PCR DETECTION AND OPERATIONAL RESPONSE TO WEST NILE VIRUS POSITIVE MOSQUITO POOLS AT SARASOTA COUNTY MOSQUITO MANAGEMENT**

CHIP HANCOCK

Sarasota County Mosquito Management, 5531 Pinkney Ave., Sarasota, FL 34233

At Sarasota County Mosquito Management, virus surveillance in field collected mosquitoes is conducted using the latest real-time PCR technology. An inexpensive, custom made assay for the QuantStudio 5 was produced in late 2016 for seasonal sur-

veillance of West Nile Virus within Sarasota County. Beginning in July of 2018, West Nile Virus positive mosquito pools were detected in our laboratory and subsequently sent to Florida Department of Health Laboratory in Tampa, FL. This method of virus surveil-

lance allowed us to detect positive mosquito pools within 2 hours of pooling, maximizing our operational response time. This is an overview of our in house detection process and our operational response to the detected presence of virus in local mosquitoes. Operational responses included: enhanced

surveillance of mosquito populations, increased mosquito pooling, increased targeting of larval habitats in the area, increased spraying for adult mosquito vectors, container abatement projects, and the use of the Code Red notification system.

## **CONTRASTING IMPORTANCE OF STATE AND IN-HOUSE LABORATORY ARBOVIRUS TESTING OF SENTINEL CHICKENS AND MOSQUITO SAMPLES**

MILTON STERLING

Lee County Mosquito Control District, Lehigh Acres, FL 33971

Mosquito-borne virus surveillance is an integral component of all mosquito control programs to monitor virus activities, human-associated mosquito-borne disease cases, and other factors to forecast or perceive variations in mosquito-borne virus transmissions. Common surveillance methods such as the sentinel chickens and adult mosquito trapping are monitoring tools available for use by the health department and mosquito control districts. Samples collected by most

mosquito control districts are not tested in-house, but by the State Health Department, which report results back to the districts. There are advantages and disadvantages associated with State-only and in-house laboratory arbovirus testing of sentinel chicken and mosquito samples. These aspects include but not limited to; results from a certified lab, cost effectiveness, turnaround time for response, erroneous results, and the reliability on a single source for results.

## **FREQUENT SUGAR FEEDING BEHAVIOR BY *Aedes Aegypti* IN BAMAKO, MALI MAKES THEM IDEAL CANDIDATES FOR CONTROL WITH ATTRACTIVE TOXIC SUGAR BAIT (ATSB)**

PRESENTED BY GUNTER C. MULLER

Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Sciences, Techniques and Technology of Bamako, Mali and Faculty of Medicine, Hebrew University, Jerusalem, Israel

*Aedes aegypti* are notoriously difficult to control since their ubiquitous man-made and natural breeding sites, in various geographical regions, include almost any receptacle that can hold water. These diurnal mosquitoes are anthropophilic, a preference that promotes their role as vectors of many arboviruses including Zika, dengue, chikungunya, and yellow fever. With the exception of yellow fever, there are no vaccines against any of these arboviruses so that use

of personal protective measures and mosquito vector control are the only means of prevention. Disease burdens in most endemic areas are not sufficiently reduced by various integrated vector management (IVM) strategies, hence there is a need for new control tools to complement the common strategies. Control by Attractive Toxic Sugar Baits (ATSB) appears to be an ideal candidate for this purpose. The results of this study support this proposition. They demonstrate that

*Ae. aegypti* in their urban environments in Mali are attracted to and frequently feed on staple diet that includes a variety of flowers, fruits and seed pods. Therefore, *Ae. aegypti* is a suitable candidate for control with ATSB.

Moreover, the experiments with ATSB, in sparse vegetation or with competitor plant attractants in rich vegetation, demonstrated that ATSB treatment can cause a drastic reduction of *Ae. aegypti* populations.

## USING MUTANT MOSQUITOES TO FIND LIFE-SAVING PERFUME

JOSHUA I. RAJI<sup>1</sup>, NADIA MELO<sup>2</sup>, JOHN CASTILLO<sup>1</sup>,  
SHEYLA GONZALEZ<sup>1</sup>, VALERIA SALDANA<sup>1</sup>,  
MARCUS STENSMYR<sup>2</sup> AND MATTHEW DEGENNARO<sup>1,\*</sup>

<sup>1</sup>Department of Biological Sciences, Florida International University, Miami, FL

<sup>2</sup>Department of Biology, Lund University, Sweden

\*corresponding author, mdegenna@fiu.edu

Detection of volatile chemicals is essential for mosquitoes to find their human hosts and spread diseases. Human odor, a blend of volatile chemicals derived from the metabolism of sweat by skin microbiota and exhaled carbon dioxide (CO<sub>2</sub>) strongly attract mosquitoes. The molecular mechanism by which mosquitoes translate host odor information into host-seeking behavior has until recently only begun to be understood. Connecting the chemical cues that attract and repel mosquitoes with their cognate genes and neural circuits will inform new strategies to control mosquito behavior. A necessary step to achieving this goal is to identify the receptors that

detect attractive volatile chemicals. We have identified and dissected in detail the role of *Ir8a* in human host-seeking by female *Aedes aegypti* Linn. mosquitoes. Our behavior assays and electrophysiology experiment showed that *Ir8a* is required for sensing acidic volatiles found in human odor, including lactic acid - a human sweat component. This study implicates *Ir8a* that enable mosquito host attraction as a molecular target for controlling mosquito population. This can inform our knowledge of formulating novel repellents to prevent mosquito bites as well as attractants to improve mosquito surveillance and population reduction strategies.

## LABORATORY EVALUATION OF TWO NEW ACTIVE INGREDIENTS FOR ATTRACTIVE TOXIC SUGAR BAITS (ATSB) AGAINST MOSQUITOES

PRESENTED BY MOLLY CLARK

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Attractive toxic sugar baits (ATSB) is a novel control method for adult mosquitoes. The active ingredient usually is a stomach poison through oral administration. Boric acid, spinosad, and other insecticides without repellent function, and several botanical oils have been tested for

the active ingredients for the ATSBs. NatureCide pest management, a product available in private and commercial home pest control (a mixture of 25.3% cedarwood oil and 12.7% cinnamon oil) and tolfenpyrad (4% plus 40% PBO) were mixed with 1% sugar as baits provided to adult female *Ae-*

*des aegypti* Linn. mosquitoes and caused significant mortality 48 hrs post treatment. Also 1% boric acid as positive control and the boric acid bait resulted in high mor-

ality, compared with the NatureCide and Tolfenpyrad. The bioassays method for Tolfenpyrad as a stomach poison needs to be further addressed.

## **AUTOCIDAL GRAVID OVITRAP (AGO) INCORPORATION WITH ATTRACTANTS FOR CONTROL OF GRAVID AND HOST-SEEKING *Aedes aegypti* (DIPTERA: CULICIDAE)**

HUI LIU<sup>1,2</sup>, DANIEL DIXON<sup>2</sup>, CHRISTOPHER S. BIBBS<sup>2</sup>, AND RUI-DE XUE<sup>2,3</sup>

<sup>1</sup>Jiangsu Provincial Disease Control and Prevention, Nanjing, 210009, China

<sup>2</sup>Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

<sup>3</sup>PRESENTED BY RUI-DE XUE

*Aedes aegypti* Linn. is a vector for multiple arboviruses including Zika, Dengue, Chikungunya and Yellow Fever. The CDC's Autocidal Gravid Ovitrap (AGO) is a new trapping method used to control container-inhabiting mosquitoes. It has been used in the area-wide management of *Aedes aegypti* in Puerto Rico, but novel modifications to existing technologies could help increase their effectiveness at controlling container-inhabiting mosquitoes. In this study, AGO traps were modified to be baited with different combinations of BG lure and octenol to increase their effectiveness

at capturing host-seeking and gravid *Aedes aegypti*. The addition of octenol to the AGO trap did not increase the number of female mosquitoes captured compared to the AGO alone. However, the AGO traps baited with BG lure caught a significantly higher number of host-seeking female *Aedes aegypti* compared to the controls and octenol. This study showed that the combination of AGO traps with BG lure potentially increases the effectiveness of AGO traps by passively collecting both gravid and host-seeking female *Aedes aegypti* mosquitoes.

## **OVER 1,600 AUTOCIDAL GRAVID TRAP (AGO) DEPLOYMENT IN ST. AUGUSTINE, 2018 AND ITS IMPACT ON CONTAINER-INHABITING MOSQUITOES**

PRESENTED BY DENA AUTRY

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Anastasia Mosquito Control District (AMCD) and two other districts across the United States were part of a study to evaluate the impact of a massive deployment of the CDC's Autocidal Gravid Ovitrap (AGO) on *Aedes* mosquito populations in 2017 & 2018. The first study left the District with many questions as it collected an abundant number of non-targets in 2017. In 2018, AMCD continued the study in other areas of the county which were

heavily populated with *Aedes* mosquitoes, the District deployed over 1,600 AGOs. The studies were done to determine the effectiveness of the AGO traps. There were six sites in the district. Three of the sites were treated with AGOs and three were control areas housing only sentinel autocidal gravid ovitraps (SAGO). The treated and control areas have SAGO traps to monitor what gets into the traps each week. AGO traps act as a leave and forget trap re-

quiring minimal maintenance. *Aedes* population were monitored with SAGO and BG Sentinel traps. Once a week three BG traps were deployed and collected for identification and count in each site and SAGO sticky cards were collected, identified, and re-

placed each week for every site. The trap deployment reduced certain number of *Aedes* mosquitoes in some spots. The study again determined to collect high numbers of non-targets but were highly liked and accepted by the citizens in the areas.

## **EFFICACY OF PLANT SAUCERS TREATED WITH RESIDUAL LARVICIDES AGAINST *Aedes albopictus* LARVAE UNDER THE SEMI AND FIELD CONDITIONS**

YONGXING JIANG

Gainesville Mosquito Control, Gainesville, FL 32601

Previous studies indicated that plant saucer is one of the most preferable breeding containers for *Aedes albopictus* Skuse in the urban environment. Conventional methods to control *Ae. albopictus* such as source reduction and adult ULV spray are either too labor-intensive or not very effective. The propose of this study was to assess the efficacy and longevity of plant saucers treated with three residual larvicides, Talstar® P Professional, Lambda 9.7 CS, and Mavrik® Perimeter at the maximum rates against *Ae. albopictus* larvae under the semi-field (shaded) and field (open) conditions. Two types of plant saucers, clay and plastic were tested in this study. Plant saucers were kept under the semi-field and field conditions after being treated with larvicides. They were subsequently brought back to the laboratory for bioassay at 1, 3-day and weekly intervals. 200 ml distilled water was added into each saucer and afterward, 20

3rd instar larvae were introduced into each saucer for bioassay. Mosquito mortality was checked 24-h post-treatment. For the clay type of saucers, under the semi-field conditions, Talstar yielded good control (>85%) of larvae *Ae. albopictus* for 21 weeks, Lambda lasted for 14 weeks, whereas, Mavrik had good control for one day and was only 60% for the first week. Under the field conditions, Talstar yielded good control for 20 weeks, Lambda yielded good control for 7 weeks. Again, Mavrik only lasted for one day. Likewise, for the plastic type of saucers, under the semi-field conditions, both Talstar and Lambda yielded good control for 18 weeks and Mavrik only lasted for one week. Under the field conditions, Talstar, Lambda, and Mavrik yielded good control up to 18, 9 and 1 week, respectively. Results from this study appear that plant saucers treated with residual pesticides can be used for *Ae. albopictus* larvae control.

## **FLYING UNDER THE INFLUENCE: IMPAIRED FITNESS OF SUSCEPTIBLE, RESISTANCE, AND FIELD STRAINS OF *Aedes Aegypti* AFTER 60 SECOND EXPOSURES TO METOFLUTHRIN VAPORS**

CHRISTOPHER S. BIBBS

Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092

Prior work with volatile pyrethroids demonstrated a variety of sub-lethal fecundity impairments that occurred in laboratory

strains of mosquito. To expand prior work, pyrethroid susceptible Orlando strain, pyrethroid resistant Puerto Rican strain, and

St. Augustine field strain of *Aedes aegypti* (L.) were all exposed to metofluthrin for 60 seconds prior to blood feeding. After a blood meal, changes in egg dispersion, egg yield, egg viability, accelerated hatching of eggs, and subsequent larval mortality in F1 generations were documented in all three strains with no significant interaction of the strain with the treatment outcomes. All strains exhibited a decline in the number of containers oviposited into from 6 to 0-1 cups. All strains also exhibited 40-50% decrease in egg yield and an additional 30% decrease in hatch viability. Orlando and St. Augustine strain retained 10-20% of eggs *in vivo* without ovipositing at all, with oc-

casional instances of eggs melanized prematurely. Approximately 20-40% of Puerto Rican and St. Augustine strain eggs hatched 12-24h faster than unexposed mosquitoes, with a resulting 20-40% mortality in the larval F1 generation. Metofluthrin appears to reduce fitness of *Ae. aegypti*, regardless of resistance phenotype, in multiple ways as a consequence of 60 seconds exposure, highlighting the potential to observe these effects in real world application scenarios. This work was a collaboration between the Anastasia Mosquito Control District, the United States Department of Agriculture, the University of Florida, and MGK/Sumitomo Chemical Company.

## ASSESSING THE EFFICACY OF OPERATIONAL MOSQUITO CONTROL PRODUCTS THROUGH FIELD TRIALS

CASEY PARKER<sup>1</sup>, DAVIELA RAMIREZ<sup>1</sup>, AND C. ROXANNE CONNELLY<sup>1,2</sup>

<sup>1</sup>University of Florida, Entomology and Nematology Department, Florida Medical Entomology Laboratory, 200 9th St SE, Vero Beach, FL 32962

<sup>2</sup>Centers for Disease Control and Prevention, Division of Vector Borne Diseases, 3156 Rampart Rd, Ft Collins, CO 80521

In recent decades, local transmission of dengue, chikungunya, and Zika have highlighted the importance of controlling *Aedes aegypti* and *Ae. albopictus* in Florida. Additionally, these species are significant nuisance species as they live in close proximity to humans and are seeking a bloodmeal during times when humans are most active. Susceptibility to insecticides is critical to effective mosquito control. CDC bottle bioassays were conducted on *Ae. aegypti* and *Ae. albopictus* and revealed resistance to both pyrethroids and organophosphates. However, many factors influence the efficacy of formulated products in the field and a laboratory assay is not sufficient to indicate field failure of a product. Through a

cooperation with Florida mosquito control programs, field trials with pyrethroids and organophosphate products were conducted against both *Ae. aegypti* and *Ae. albopictus* at operationally-relevant application rates with three mosquito control programs. Field trials with Fyfanon® against *Ae. aegypti* in Pasco County resulted in rapid (1 hr) mortality that remained constant at the 24 hr mortality reading. Deltagard® trials in the same county with the same populations did not result in the same levels of mortality and some recovery was observed at the 24 hr mortality reading. Results from this study show similarities in mortality trends observed in the CDC bottle bioassay and field trials.

# EDITORIAL

# ACKNOWLEDGEMENTS

The following scientists have provided valued assistance in reviewing articles for this issue. Names followed by an asterisk (\*) are of individuals who have reviewed two or more manuscripts. A special thank you is given to these scientists by the editors. The editor also acknowledges V. Aryaprema, C. Efstathion, M. Farooq, E. Khater, and M. Miah as the Guest Editors for 1 or 2 manuscripts' peer-review and editing process. Also, the editor thanks Charolette Hall for editorial assistance and proofreading.

V. Aryaprema\*

K. Blore

C. Boohene

M. Debboun

C. Efstathion\*

A. Faraji

M. Farooq\*

Y. Jiang

E. Khater\*

P. Koehler

N. Indelicato

C. Lesser

A. Lloyd

K. Lucas

M. Miah\*

G. O'Meara

W. Qualls

H. Sanchez-Arroyo\*

I. Unlu\*

G. Vasquez

Y. Wang

A. Wike

K. Yanchula





# JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

## INFORMATION FOR CONTRIBUTORS

The Journal of the FMCA encourages the submission of unpublished manuscripts in the field of biology and control of mosquitoes and mosquito-borne diseases.

Manuscripts in MS Word or Rich Text Format should be sent to the Editor, JFMCA, Dr. Rui-De Xue, Anastasia Mosquito Control District, 120 EOC Drive, St. Augustine, FL 32092, USA by e-mail attachment at [xueamcd@gmail.com](mailto:xueamcd@gmail.com). Each manuscript will be sent to 2 or 3 authorities for peer review. Their comments and recommendations remain anonymous and are forwarded to the authors. The Editorial Board of the Journal serves as an adjudication panel for resolving conflicts between authors and the editors.

Manuscripts require double space throughout, including references and indented paragraphs. A title page containing the corresponding author's complete mailing address, telephone and fax numbers, and email address should be included, as well as the name and affiliations of all co-authors. Each article must be accomplished by an abstract not longer than 3% of the paper and a short title of not more than 40 letters to serve as a running head. Five important key words are required. The paper should be divided as follows: abstract, key words, introduction, material and methods, results, discussion, acknowledgments, and references cited. References should conform to the style presented in this issue.

Tables should be used sparingly and self-explanatory. Each table should be double spaced on its own page and all acronyms should be explained in a footnote. Only high quality, computer-generated graphs will be accepted. Figure keys should be included on the figure itself. Photographs should be glossy prints with sharp focus and good contrast.

The journal accepts the submission of operational notes or scientific notes. The notes may contain 1 or 2 tables and or illustrations, with acknowledgements included in the last paragraph of the text. There should be an abstract and key words. No section headings are needed.

Following peer review, authors are required to submit their revised manuscript in electronic format. Authors are expected to read proofs carefully, make corrections, answer queries, and return proofs promptly to the editors.

# FLORIDA MOSQUITO CONTROL ASSOCIATION

The mission of the FMCA ([www.floridamosquito.org](http://www.floridamosquito.org)) is to promote effective and environmentally sound control of disease-transmitting and pestiferous mosquitoes and other arthropods of public health importance, develop and enhance public interest, awareness, and support for the control of mosquitoes, and provide for the scientific advancement of members through our meetings, training and education. The FMCA is a non-profit, technical, scientific and educational association and publishes the Journal of The Florida Mosquito Control Association in the furtherance of these objectives.

## BOARD OF DIRECTORS, 2018-2019 OFFICERS

### **Andrea Leal, President**

Florida Keys Mosquito Control District  
5224 College Road  
Stock Island, Key West, FL 33040

### **Donnie Powers, President Elect**

FMC Corporation  
(205) 641-1157

### **James Clauson, Vice President**

Beach Mosquito Control District  
Panama City Beach, FL 32407  
(850)233-5030

### **T. Wayne Gale, Immediate Past President**

Lee County Mosquito Control District  
15191 Homestead Road  
Lehigh Acres, FL 32971  
(239)694-2174

### **Martin O'neil, Executive Director**

ExecutiveDirector@floridamosquito.org  
1-866-Go4-FMCA

## REGIONAL DIRECTORS

### **Austin Horton, Northwest Region**

Gulf County Mosquito Control  
1001 10th Street  
Port St. Joe, FL 32456  
(850)227-1401

### **Peter Jiang, Ph.D. Northeast Region**

City of Gainesville Mosquito Control  
405 NW 39th Avenue  
Gainesville, FL 32169

### **Katie Williams, Southwest Region**

Manatee County Mosquito Control District  
2317 2nd Avenue West  
Palmetto, FL 34221  
(941)722-3720

### **Sherry Burroughs, Southeast Region**

Indian River Mosquito Control District  
5655 41st Street  
Vero Beach, FL 32967-1905

### **Wendy DeCorah, Industry Representative**

ADAPCO  
550 Aero Lane  
Sanford, FL 32771  
(407) 328-6576

### **Barry Alto, Member-at-large**

University of Florida/FMEL  
200 9th Street SE  
Vero Beach, FL 32962  
(772)778-7200

### **John Magee, Commissioner Representative**

South Walton Mosquito Control District  
P.O. Box 1130  
Santa Rosa Beach, FL 32459  
(850) 267-2112