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

JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

VOLUME 68, 2021

JOURNAL OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

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Formatting, design, and layout by E.O. Painter Printing Company, P.O. Box 877, De Leon Springs, FL 32130

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2019-2020 THE FMCA PRESIDENTIAL ADDRESS

DONALD POWERS
FMC Corporation

Serving as President of the Florida Mosquito Control Association has been an honor, a pleasure, and a challenge. I take great pride that the FMCA has navigated the COVID-19 crisis as well as the adjustments and changes we have made in the management of the association. The past year has been a period of transition and change. Change is never easy and rarely is an easy choice the correct one. As we have moved through this year we have evolved, learned a tremendous amount, and become a stronger community. As a member, I want to thank everyone for membership and participation in FMCA.

The Board of Directors identified key initiatives necessary for the sustainability of our association. Our focus centered on creating an environment that encourages volunteerism and cultivates professional development, improving communication and cohesion through all forms of media, and organizing the association finances and records.

We must establish the conditions from which volunteerism thrives. Several new volunteers have commented that in past years they wanted to participate but did not feel as if there was an opportunity. Without active participation at all levels, our association will never make progress. Everyone has skills, talents, and perspectives that they can contribute to our community. The more diversity we have, the more successful we will be together. I challenge every member to take an active role in our organization.

As I have mentioned before, we have done our volunteers a disservice. In the past we have allowed them to take on workloads that were not reasonable, fair, and ultimately limited their opportunity for success. This unfairly left certain volunteers open to scrutiny, criticism, and liability. In 2020 we consolidated the business functions of the association with licensed and bonded firms to alleviate our volunteers of these responsibilities. Checks and balances are in place to provide volunteers with full oversight and transparency.

I challenge program Directors and academic advisors to consider service for employees of all levels. If you have young professionals, employees, or students please utilize FMCA as a leadership and professional development opportunity. Use volunteer roles as progressive training and you will likely discover talents and aptitudes that will return value to your district or organization. While serving our association, volunteers will grow as individuals, employees, and become better representatives of the mosquito control community.

Financial organization has been a core focus for the Board and Finance Committee. We initiated a comprehensive audit of all FMCA expenses and records. This, along with the contributions of our new Executive Director, are bringing our finances in line with standard business and accounting practices. The Finance Committee and FMCA Board of Directors are focusing on checks and balances that will increase transparency and confidence in our association. I want to recognize John Magee (ad hoc Treasurer), the Finance Committee, and everyone who volunteered their time in coordinating the audit process.

I did not get to spend as much time focusing on FMCA communications as I had hoped. Ultimately, all FMCA publications, traditional and social media, and communi-

cations will be aligned and maximize value to our membership, readership, and advertisers. Through the diligent work of our Public Information Committee our association increased its presence and dissemination of content significantly. I would like to take a moment to recognize Jillian Meek and Michael Mut for their contributions and professionalism.

As a final challenge, I want to speak directly to the Young Professionals. You are passionate about the advancement of science and developing your career networks. We recognize our senior leadership as they are preparing to retire. I believe this is an underutilization of their talents, experience, and wisdom. Please engage, meet, and interview these senior leaders as well as their co-workers and friends. These interviews can produce articles and content that will be greatly appreciated by our membership. We can't fully comprehend or appreciate the value we are losing by letting this knowledge slip away. These opportunities can yield immense value in capturing more of our institutional knowledge and collective memory of the FMCA community. As young professionals, this would be an excellent opportunity that would enrich you personally while returning value to our association.

If you have a concern or problem, please bring it before the Executive Director, your regional representative, or any FMCA representative. Please do this from a solution-oriented approach where we can discuss problems, gather all the information, evaluate impacts of each option, and everyone is part of the solution. We are all here to do what is best for the membership of our association.

It has been an honor to serve as President of the FMCA and I am better for having done so. In closing, we must conduct ourselves with respect, professionalism, and integrity as we focus energy and efforts on the mission of the Florida Mosquito Control Association.

We must keep FMCA moving forward.

Donnie Powers

WHAT MAKES A VECTOR A VECTOR, AND WHY IS THAT IMPORTANT?

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ABSTRACT

Mosquitoes and other arthropods can transmit pathogens that currently cause millions of cases of illness and over 700,000 deaths annually. For most of these, the most efficient prevention is mosquito (or vector) control. However, only a small number of mosquito species are responsible for pathogen transmission, and different species are important for different pathogens. Because mosquito (vector) control tends to be focused on specific species, it is critical to ensure that the control efforts are directed at the species that are actually involved in pathogen transmission in the real world. Therefore, it is important to understand what makes a vector a vector and the various factors that affect the ability of a potential "vector" to actually transmit a pathogen.

Key Words: Vector, Virus, Control, Disease, Mosquito

Malaria, dengue, Zika, chikungunya, yellow fever, tick-borne encephalitis, and Lyme disease are but a few of the diseases caused by pathogens transmitted by mosquitoes and other arthropods. These pathogens cause millions of cases of disease and over 700,000 deaths each year (World Health Organization 2021). Unfortunately, licensed vaccines are not available for most of these diseases, and the only method of preventing them is to reduce, or hopefully eliminate, the vector population. Mosquito Control Departments (or Mosquito Control Districts) or their equivalents have been established all over the world in an attempt to not only reduce pest mosquitoes, but more importantly, to reduce the risk of transmission of pathogens causing disease in humans and domestic animals. From here on, I may only use "mosquito" to represent all potential vectors, but the reader should remember that what I am saying also applies to sand flies, ticks, and other potential vectors.

Unfortunately, there is no simple procedure that kills all mosquitoes. Like with a vaccine, each type of control is generally directed at some specific species or group of species of mosquitoes. Some controls are directed at larvae, while others are directed at the adults. The controls are applied to different habitats and at different times of day, depending on which mosquito is the target for that particular control. Some mosquitoes are diurnal and are only active during the day. Therefore, spraying at night would have very little effect on them. Others are nocturnal and are only active at night, so spraying during the day would have very little effect on them. While still others are primarily crepuscular and are primarily active at dusk or dawn, so spraying during bright sun or late at night may have little effect on these species. Therefore, depending on the target of the control, pesticide application would be applied at different times of day. Some methods are species specific. For example. release of sterile male *Ae. aegypti* may be helpful controlling future outbreaks of Zika, dengue, or chikungunya, but would be worthless for preventing West Nile. Similarly, larval habitats differ by mosquito species. For example, the procedures used to control larval *Aedes taeniorhynchus* (Wiedemann) may have little or no impact on *Culex quinquefasciatus* Say, despite the fact that Altosid® was effective against both species (Floore et al. 1991). Similarly, adult spraying may be more efficient at controlling *Aedes vexans* (Meigen)

than *Culex tarsalis* Coquillett, even when they are co-located as adults (Gujral et al. 2007). Therefore, the control procedure needs to be directed at the species that needs to be controlled, not at "mosquitoes" in general.

Despite there being >3,500 different kinds of mosquitoes (Harbach 2013), only a relatively few are pests of humans, and only a very few are involved in pathogen transmission. Even more importantly, the mosquitoes that transmit one pathogen may not be able to transmit other pathogens. For example, the primary vectors of malaria, West Nile virus (WNV), and Zika virus (ZIKV) are completely different, and the important vectors of any of these are essentially unable to transmit either of the other two pathogens. Various *Anopheles* species are the primary vectors of human malaria, while various *Culex* species (primarily, *Culex nigripalpis* (Theobald), *Culex pipiens* (L), *Cx. quinquefasciatus,* and *Cx. tarsalis*) (Goddard et al. 2002; Andreadis 2012) are the principal vectors of WNV in the U.S. In contrast, essentially only *Aedes aegypti* (L.) is important as a vector of ZIKV. Although numerous species of mosquito in addition to *Ae. aegypti* have been shown in the laboratory to be competent vectors of ZIKV (Azar et al. 2017; Ciota et al. 2017; Dibernardo et al. 2017; O'Donnell et al. 2017), these other species are unlikely to be involved in transmitting ZIKV in the real world. Because in most parts of the world ZIKV is an anthroponotic virus, only humans can serve as a source of this virus for mosquitoes. Therefore, in order to transmit ZIKV, the same individual mosquito needs to feed on a viremic person to pick up the virus, and then needs to feed on a second human sometime later to transmit the virus. While many species readily feed on humans, very few preferentially feed on humans and thus it would be extremely unlikely for a single individual mosquito to take two separate blood meals on a human. That is why, despite there being >5,000 reported imported cases of Zika infection in the U.S., with >1,000 occurring in areas where *Aedes albopictus* (Skuse) is one of the primary pest mosquitoes (CDC 2021), no locally transmitted cases were detected in any area where *Ae. aegypti* were not a known pest.

Because bites from non-vector mosquitoes raise people's awareness about mosquitoes and the need to take precautions, merely controlling "mosquitoes" may actually have detrimental effects concerning disease suppression. As WNV spread across the U.S. in 2003, a study found that in two areas with similar demographics, more intensive mosquito control was inversely related to the amount of West Nile disease detected (Gujral et al. 2007). This unanticipated effect was probably due to intensive control of *Ae. vexans*, a severely painful and annoying mosquito that does not transmit WNV in the real world, but only limited control of *Cx. tarsalis*, the most important vector species in the area (Goddard et al. 2002; Turell et al. 2002). There were a lot of television, radio, and newspaper warnings at the time to avoid mosquitoes, apply repellants, and to protect yourself from mosquito bites to reduce your risk of becoming infected with this new virus. However, in areas with normal mosquito control, there were still sufficient *Ae. vexans* biting so that people were concerned and used various methods to reduce mosquito biting, e.g., applied repellants and wore clothing that protected skin from mosquito bites. This reduced the number of bites from *Cx. tarsalis*, and therefore the amount of transmission of WNV. However, in areas with the more intensive control, *Ae. vexans* populations were greatly reduced. The people living there had minimal detectable mosquito bites and were thus not as concerned about the need to protect themselves from mosquitoes. Because of this, there were many more bites from *Cx. tarsalis*, and thus many more cases of disease caused by WNV.

So, what makes a vector a vector, or more importantly, what makes a vector an important vector in a particular area? The mere isolation of a virus from a mosquito does not mean that the species is a vector of that virus. If the mosquito had recently fed on a viremic host, the mosquito would contain both infectious virus as well as viral RNA, even if that species was unable to become infected with or to transmit that virus. That is why mosquito species need to be tested to determine if they are competent vectors of

a particular pathogen. Obviously, if the species is not a competent vector, i.e., is unable to become infected or to transmit virus after oral exposure to the virus, then that species is not likely to be an important vector. However, different geographic populations of a mosquito species can differ significantly in their vector competence for a particular virus. For example, *Ae. vexans* from the southeastern U.S. are moderately efficient vectors of Rift Valley fever virus (RVFV) (Turell et al. 2013), while those from the northwestern U.S. or southern Canada are virtually incompetent (Turell et al. 2010, Iranpour et al. 2011). Because *Ae. vexans* readily feeds on large mammals, it might be an important vector in the southeastern U.S., but would be much less important in the northwestern U.S. There are numerous other examples where geographic populations differ greatly in their vector competence for a variety of viruses including chikungunya virus (CHIKV) and *Ae. albopictus* (Tesh et al. 1976), dengue virus (DENV) and *Ae. aegypti* (Ye et al. 2014), and western equine encephalitis virus and *Cx. tarsalis* (Hardy et al. 1976). Therefore, not only is the vector competence of a potential vector species important, but the competence of the local population of that species is important. However, just because a particular species is competent in the laboratory may not be sufficient. For nearly all outbreaks of chikungunya, *Ae. aegypti* has been the most important vector. Although the A226V amino acid substitution in the E1 envelope glycoprotein that enhances the ability of *Ae. albopictus* to transmit CHIKV has been cited as the reason for the 2005-2007 outbreaks of chikungunya that were driven by *Ae. albopictus* (Tsetsarkin et al. 2007, Riccardo et al. 2019), this mutation developed well into the outbreak. It is more likely that an outbreak involving *Ae. albopictus* selected for a strain of virus even more efficiently transmitted by this species than that the mutation allowed *Ae. albopictus* to serve as the vector. It is possible that in areas where *Ae. albopictus* has served as a significant vector, other possible blood sources, particularly dogs, may not have been present in sufficient numbers to inhibit feeding on humans. A previous study showed that *Ae. albopictus* was already a highly competent vector of CHIKV, even without the A226V mutation. When numerous geographic populations of both *Ae. aegypti* and *Ae. albopictus* were allowed to feed concurrently on the same viremic monkey, every one of the 10 geographic strains of *Ae. albopictus* was more susceptible than any of the seven strains of *Ae. aegypti* (Turell et al. 1992). Why then is *Ae. aegypti*, which in the laboratory is a less efficient transmitter of CHIKV than *Ae. albopictus*, normally a more important vector of CHIKV? Remember, CHIKV is an anthroponotic pathogen, and as such, the vector needs to feed twice on a human in order to be able to transmit CHIKV. It is well known that most populations of *Ae. aegypti* preferentially feed on humans (Scott et al. 1993), but *Ae. albopictus* tend to be more opportunistic feeders (Richards et al. 2006). In addition, while most mosquito species tend to obtain nourishment from nectar after a blood meal, *Ae. aegypti* tend to take multiple blood meals on humans during each gonotrophic cycle, thus greatly increasing its contact with humans and its ability to become infected and then transmit an anthroponotic virus (Scott et al. 1997; Costero et al. 1998). Taking of multiple blood meals per gonotrophic cycle further enhances vector competence as the stretching of the midgut due to ingestion of blood appears to enhance the development of a disseminated infection (Armstrong et al. 2020).

For most arboviruses, feeding preference of the potential vector is critical. Mosquitoes that preferentially feed on birds would be very poor vectors of CHIKV, DENV, or RVFV, as these are all viruses that affect and replicate in mammals. Similarly, mosquitoes that preferentially feed on mammals would be poor maintenance vectors of WNV, eastern equine encephalitis virus or western equine encephalitis virus as even though these viruses produce disease in various mammals, they do not produce a sufficient viremia in mammals to infect a mosquito. However, mosquitoes that feed on both mammals and birds are dangerous as they can serve as a bridge vector, picking the virus up from an

infected bird and transmitting it to a susceptible mammal. Even if a particular species is highly competent and feeds on the appropriate host, if it is present in low numbers, then it would not likely be important. To be important, the potential vector needs to be competent, feed on the appropriate vertebrate hosts, and occur in sufficiently high numbers to serve as a vector.

When controlling mosquitoes or other vectors for disease suppression, it is important to know what the potential vectors are in the area. Which species have been shown to be able to transmit the pathogen? Which species feed on the appropriate host? Which species are occurring (or are predicted to occur by environmental predictors, e.g., tides, rainfall, etc.) in sufficient numbers to be a problem? Once these potential vectors have been identified, they should be prioritized for control based on how likely they are to play a role in pathogen transmission. Remember, killing the wrong mosquito may actually make the disease situation worse.

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CONSIDERATIONS OF MORPHOLOGIC OBSERVATIONS OF MOSQUITO SPECIES FROM IDENTIFYING COMPLETE SAMPLES IN PANAMA CITY BEACH, FLORIDA

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Subject Editor: Rui-De Xue

ABSTRACT

In Panama City Beach, Florida, thirteen mosquito species have been recently registered into public health data banks over the span of 7 years [2014-2020], ten species within their published geographic range and three species outside of their noted geographic range. The underreporting is likely due to past identification practices of sub-sampling and aliquoting surveillance collections while only recording the top-most three abundant species for control application thresholds. However, these thirteen species have not been recorded in this area by public health operations up until their respective record timelines. Timelines of identification, species specific character states, the dynamic of identifying similar species and alternate identification methods are discussed. As of 2020, 10 genera and 50 species within Diptera: Culicidae are recorded in Panama City Beach, FL, U.S.A.

Key Words: Surveillance, identification, taxonomy, sub-sampling, character state, dichotomy

INTRODUCTION

In Panama City Beach, FL, the past traditional role of identification was to record the three most abundant mosquitoes in an aliquot sub-sample protocol, where collections of mosquitoes were not fully examined and only a small proportion of the actual collections was utilized for the threshold control protocols at that time. Without aliquot subsampling thirteen mosquito species have been cataloged in Bay County since 2014, where the past years database did not include mosquito species even within their natural geographic range (Table 2). These mosquito species are now recognized as occurring in Bay County, FL, even if the species are in low abundance from seasonal surveillance col-

Table 1. Descriptions of district sampling locations by site name, year placed, surveillance methods: light trap (L) canopy trap (C) gravid trap (G exit coop trap (E) and aspiration resting box trap (A), habitat type and geological locations of each surveillance sites.

Site Name	Date Placed	Surveillance Type	Habitat	Latitude	Longitude
St. Andrews	1998	L	Rural	30.13426	-85.735
Camp Helen	1998	L,G	Rural	30.27351	-85.9914
Pirates Cove	1998	L,G,A	Suburban	30.26745	-85.9768
14th Street	2006	L,C,E,G,A	Suburban	30.24777	-85.9315
Lakeside	1998	L,G,A	Suburban	30.22536	-85.8786
Frank Brown	2005	L,G,A	Suburban	30.22999	-85.8741
Surfside	2005	L,G,A	Suburban	30.20593	-85.8534
Racoon River	1998	L,G,A	Rural	30.19261	-85.8293
Arnold Highschool	2005	L,G,A	Suburban	30.20487	-85.8104
Treatment Plant	1998	L,C,E,G,A	Rural	30.21764	-85.8519
Bayside	2005	L,G	Rural	30.20214	-85.8613
Ed's Sheds	2003	L,C,E,G,A	Suburban	30.19035	-85.777
Navy Base	2006	L	Suburban	30.18129	-85.7552
Half Hitch	2005	L,G,A	Suburban	30.16317	-85.7571
Sanctuary Beach	2007	L,G,A	Rural	30.14318	-85.7144

lections. Proper identification of mosquito species is paramount when planning public health mosquito and vector control applications. Correctly cataloging mosquito species assists in strategizing the suppression of pestiferous and pathogenic mosquito species. Invasive mosquito species can only be correctly recognized by the knowledge base of the identifier particularly when invasive species have similar anatomical character states as native species which could be misidentified in collections outside their reported geographic range (Riles et al. 2017). Educating public health officials is of the utmost importance concerning the identification of mosquito species especially with new introductions into the United States (Shroyer et al. 2015, Blosser et al. 2016, 2017, Reeves et al. 2020), the state of Florida (Smith et al. 1988, Darsie et al. 2004, Smith et al. 2006, Shin et al. 2016, Riles et al. 2017) and the migration of reported mosquito species as they move from one county to the next (Smith et al. 2020, Connelly and Riles 2020). Current literature for identification is just as important as proper surveillance methods. In 1981 Richard Darsie and Ronald Ward published, "Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico". This has served as the primary source for mosquito species identification in North America with the most recent publication in 2005 which leaves a gap of current information concerning distribution of mosquito species of naturalized and invasive mosquito species and novel anatomical character states that have been discovered (Harrison et al. 2016). The state of Florida's dichotomous key for mosquito identification, "Keys to the Adult Females and Fourth Instar Larvae of the Mosquitoes of Florida (Diptera: Culicidae)" was last updated in 2009 (Darsie and Morris 2003) with the introduction of *Culex coronator* into the state (Smith et al. 2006) and needs to be updated to reflect the recent introductions of species into Florida. In 2012, Nathan Burkett-Cadena published, "Mosquitoes of the Southeastern United States". This is the first integrated full-color mosquito identification guide with added bionomic information and updated distribution maps (Burkett-Cadena 2012). In 2016, Bruce Harrison and Brian Byrd published "The Mosquitoes of the Mid-Atlantic Region: An Identification Guide", this publication was a necessary update for the region including couplets with novel anatomical character states for genera including *Aedes*, *Mansonia*, and *Culex* (Harrison et al. 2016). This guide includes counties that border northern Florida and can be considered a useful guide for migratory corridors with Alabama and Georgia.

Thirteen mosquito species have been cataloged in the database at Beach Mosquito Control District in Panama City Beach, FL. Twelve of these species have been published (Darsie and Morris 2002, Riles et al. 2017, Connelly and Riles 2020) and one species is mentioned here for the first time. Gross level identification practices such as subsamples and aliquots are the known preferred protocol for the identification process in public health agencies, although identifying whole samples enables the identifier to know the true diversity of the area surveyed. Identification timelines and practices are discussed below concerning the dynamic of identifying similar species.

MATERIALS AND METHODS

Beach Mosquito Control District located in Panama City Beach FL samples mosquitoes using: 1) Center for Disease Control (CDC) light traps (Model 1012 John W Hock Gainesville, FL), baited with pressurized carbon dioxide and octenol; 2) Center for Disease Control gravid traps (John W Hock Gainesville, FL), 3) BG Sentinel 2 traps (BioGents), 4) specialized acrylic light traps (Manufactured on site BMCD unpublished data); 5) aspirators, and 6) canopy traps (Model 1012 [modified], John W Hock, Gainesville, FL). Sixteen CDC light trap sampling locations in Panama City Beach, FL have been statically placed since 1998 through 2007 (Table 1). These sites have been sampled twice per week from February through November each seasonal application year. Three sampling locations are set for arbovirus surveillance and are

Table 2. Descriptions of go to morphological character states used in identification of mosquito species that are similar. Species of interest, similar species and character states are described.

Genus	Species	Similar Species	Character	Character	Character
Culex	*interrogator*	*Cx. restuans*	Wing measurement	Scutum spots	Size
Culex	*pilosus*	*Cx. erraticus*	Vertex scales	Integument	Sternites
Culex	*peccator*	*Cx. erraticus*	Vertex scales	Integument	Sternites
Aedes	*japonicus*	*Ae. aegypti*	Lyre like scales	Palps	Hind tarsomere 5
Aedes	*c. mathesoni*	*Ae. c. canadensis*	Hind tarsomeres 1-2	Hindtarsomere 3	Hind tarsomere 5
Aedes	*tormentor*	*Ae. atlanticus*	Compound eye	Occipital scales	Scutum scales
Aedes	*dupreei*	*Ae. infirmatus*	Size	Subspiracular scales	Scutum scales wider
Psorophora	*horrida*	*Ps. ferox*	Scutum scales	Abdomen tergum I scales	N/A
Anopheles	*perplexans*	*An. punctipennis*	Wing subcostal pale spot	Wing subcostal dark spot	N/A
Mansonia	*titillans*	*Ma. dyari*	Flagellomere 1	Abdomen tergum VII	Spiniform

monitored using sentinel chickens since 1998, these sites are equipped with canopy traps (2006) set at 9 meters vertically and exit coop traps. Eleven CDC gravid trap locations have been monitored since 2005 where each site is equipped with resting box traps that are aspirated and sampled once per week. Biogents Sentinel traps have been utilized since 2014 to monitor *Stegomyia* mosquitoes. Mosquitoes are knocked down with carbon dioxide gas for 1 hour and then each net is processed and identified by site and collection net. Mosquitoes are identified using current dichotomous identification keys, combining character states and distribution zones from all three keys (Darsie and Ward 2005, Burkett-Cadena 2012, and Harrison et al. 2016), and have been viewed using a Motic SMZ-161 stereomicroscope where mosquitoes were separated by sex, genera, and species. *Culex interrogator* wing length and wing cell were measured by using calipers (BioQuip, Rancho Dominguz, CA) to distinguish from populations of *Cx. restuans* and *Cx. pipiens quinquefasciatus*. Males are not speciated or reported here although they are counted and stored into the district's database. All data is entered into the district's database software MapVision Gen 2 (Leading Edge, Inc).

RESULTS

Aedes (*Hulecoeteomyia*) *japonicus japonicus* (Theobald, 1901).

Darsie and Ward (2005) describe *Aedes j. japonicus* with yellow scales on the scutum with a lyre-shaped marking on a black scaled background, where this species can be separated from *Aedes aegypti* (L.) from 1) the median longitudinal stripe of yellow scales on the scutum being absent; 2) the presence of basal traverse pale bands on terga III-VII and 3) the hindtarsomere 5 with pale scales. Harrison et al. 2016 describes separating *Aedes j. japonicus* from *Stegomyia* mosquitoes 1) the scales on the lobes of the scutellum are long, narrow; 2) palpus covered in black scales only; 3) hindarsomeres 1-3 have broad basal white bands with hindarsomere 4 scaled black (with

a rarely seen small dorsobasal pale spot) and 5) tarsomere 5 entirely scaled in black (Table 2). *Ae. j. japonicus* abundance in this region is minimal (n=21; 2014-2020) (Table 1).

Mansonia titillans (Walker) 1848 & *Mansonia dyari* (Belkin, Heinmann and Page, 1970).

The characters utilized to determine the correct identification of *Mansonia titillans* are 1) the antennal flagellomere 1 with a medial patch of broad black scales (Harrison et al 2016) and 2) the abdominal tergum VII with a long transverse row of short black spiniform setae beneath the scales of the posterior margin (Darsie and Ward 2005, Burkett-Cadena 2012). *Ma. dyari* were collected, identified (Harrison et al 2016). The character states of the absence of scales on the antennal flagellomere 1 (Harrison et al. 2016) and the lack of spiniform setae beneath the scales of the posterior margin of the abdominal tergum VII are what determined and verified these specimens from intermixed populations of *Ma. titillans* (Table 3).

Culex (*Melanoconion*) *peccator* (Dyar and Knab, 1909) & *Culex* (*Melanoconion*) *pilosus* (Dyar and Knab, 1906).

Culex erraticus is a common mosquito observed in Bay County, FL. (n=25,788 F, 2014-2019). At gross levels, the *Melanoconion* subgenus within *Culex* can be grouped and misidentified based on common anatomical characters states of size, occipital broad scales bordering the eye, and mesepermial integument shading (Darsie and Ward 2005, Burkett-Cadena 2012, Harrison et al. 2016). *Cx. erraticus* can be separated from other subspecies by 1) the vertex with several rows of broad round scales behind the eye and 2) a distinct patch of white scales in the middle of the mesepimeron (Darsie and Ward 2005). Harrison et al. 2016 describes separating *Cx. peccator* and *Cx. pilosus* from *Cx erraticus* where 1) the mesepimeron is without scales and 2) the vertex is completely covered in flat round scales. Further separation where *Cx. peccator* has 1) the mesokatepisternum with an upper patch of 5 or more scales and 2) the mesepimeron is present with a dark angalate ventral integument that has the posterior-dorsal tip adjacent to the metathoracic spiracle. *Cx. pilosus* only has only 2-3 broad white scales in the upper patch on the mesokatepisternum with the dark ventral integument on the mesepimeron with the dorsal margin reaching the posterior border of the mesepimeron well below the metathoracic spiracle (Harrison et al. 2016) and Burkett-Cadena (2012) describes *Cx. pilosus* abdominal sternites with distinct basal and dark apical bands as *Cx. peccator* is described as having mostly pale abdominal sternites that have a darker apical edge (Table 2).

Culex (*Culex*) *interrogator* (Dyar and Knab, 1906).

At gross levels, *Cx. interrogator* can be confused with *Cx. restuans* (Theobald) and/

Table 3. A time line of mosquito species added to district databases from correctly identifying species 2014-2020. Identified mosquito species, life stage, method of surveillance and amount collected over time is described below.

Species	Time	Life Stage	Trap Type	Amount
Aedes japonicus	2014-2020	Adult(F)	CDC Light Trap	21
Ae. tormentor	2014-2020	Adult (F)	CDC Light Trap	36
Psorophora horrida	2014-2020	Adult (F)	CDC Light Trap	29
Mansonia titillans	2014-2020	Adult (F)	CDC Light Trap	264
Toxoryhnchities rutilus	2016-2019	Adult (F)	BG Sentinel 2	2
Orthopodomyia signifera	2016-2020	Adult (F)	CDC Canopy Trap	13
Culex pilosus	2017-2020	Adult (F)	CDC Light Trap	1171
Cx. peccator	2017-2020	Adult (F)	CDC Light Trap	11
Aedes dupreei	2017-2020	Adult (F)	CDC Light Trap	18
Cx. interrogator	2018-2020	Adult (F)	CDC Gravid Trap	125
Anopheles perplexans	2019-2020	Adult (F)	CDC Light Trap	43
Mansonia dyari	2019-2020	Adult (F)	CDC Light Trap	11
Ae. canadensis mathesoni	2020	Adult (F)	CDC Light Trap	2

or *Cx. p. quinquefasciatus* (Say) (Shin et al. 2016) due to similarities in morphological character states. The specific anatomical characters that set this invasive *Culex* species apart from similar species are 1) size, 2) wing length and, 3) wing cell (Carpenter and La Casse 1955). *Cx. interrogator* is described by Darsie and Ward (2005) as a 1) small species with a total wing length less than 2.8 millimeters, 2) without a pair of pale spots located at the submedian middle of the scutum and the 3) wing cell (R_2 3.0–4.0 length of vein R_{2+3}) (Table 2). The dorsal view can assist in the identification of the wing when using calipers to measure lengths of the wing vein and the whole wing. Based on morphological character states described, *Cx. interrogator* has been collected in a series of weekly CDC light and gravid trap collections (n=125 F) and recorded in the district database May 2018 through September 2020 (Table 3).

Psorophora (*Janthinosoma*) *horrida* (Dyar and Knab, 1908).

A population of female *Psorophora horrrida* (1n=16F, 2n=3F) were observed intermixed with collections of *Psorophora ferox* (von Humboldt) (1n=121F, 2n=6F). At gross levels, these two *Janthinosoma* species can appear to be similar, whereas the hind tarsomeres T_{a4} and T_{a5} are scaled fully white (Carpenter and LaCasse 1955, Darsie and Ward 2005). Light trap fan blades can damage specimens where scutum character states cannot be used to verify species and secondary character states are required to make determinations. Morphological character states 1-6 as described by Harrison and Whitt (1996) are extremely helpful when separating *Ps. horrida* from abundant collections of *Ps. ferox* where morphological characters 2 and 3 were the most beneficial in our identification: 1) lateral scutal scaling on *Ps. ferox* is a mixture of gold and brownish-purple versus *Ps. horrida*'s lateral scutal scales are a creamy, yellowish toward white and 2) abdominal tergum I scaling on *Ps. horrida* is creamy-white versus *Ps. ferox* scales are distinguishably purple (Table 3).

Anopheles (*Anopheles*) *perplexans* (Ludlow, 1907).

Two female *Anopheles perplexans* were collected from a CDC light trap and cataloged as a county record (Riles and Connelly 2020). The dark scaled palpi with the wing vein R_{4+5} and Cu with dark scales only defines this *Anopheline* species along with the determining character states of wing spots, where the subcostal spot is reduced to less than 1/3rd the length of the preapical dark spots versus *An. punctipennis* (Say) subcostal pale spot ½ or more length of the subapical dark spot (Darsie and Ward 2005) (Table 2). Forty-one specimens have been collected, identified, and cataloged in district databases from April 2019 through October 2020 (Table 3).

Aedes (*Ochlerotatus*) *tormentor* (Dyar and Knab, 1906).

Roberts and Scanlon described separating the females of *Aedes tormentor* and *Aedes atlanticus* in 1979. Since these descriptions public health identifiers have included both species together as a*tlanticus/tormentor* or *tormentor/atlanticus* and has been described in dichotomous keys as such up until Sither (2013) described separating females of both species by molecularly defining differences of flat black occipital scales extending to or not extending to the compound eye. In Harrison (2016) these differences are now described in a dichotomous key where the character states: 1) Black lateral occipital flat scales on the head extend forward to reach the eye (*Ae. atlanticus*) versus black lateral occipital flat black scales on head do not reach the eye due to 2-3 rows of narrow white scale bordering the eye (*Ae. tormentor*) and 2) the scutum has a median longitudinal pale stripe with equal symmetry concerning the width anteriorly and posteriorly (*Ae. atlanticus*) versus the stripe being narrow at the posterior end (*Ae. tormentor*) can easily separate the two like species (Table 2). Since April 2014, *Ae. tormentor* has been determined by using these novel morphological character states (n=36F) (Table 3).

Aedes (*Ochlerotatus*) *dupreei* (Coquillett, 1904).

Aedes dupreei, since November 2017, have been separated from collections

(n=18) mixed with *Aedes infirmatus* (Dyar and Knab) (Table 1). This smaller similar species identification can be considered ambiguous at gross level identification (*Ae. infirmatus* 2014-2020 N=29,383) where *Ae. infirmatus* subspiracular area has scales marginally placed between the hypostigmal area and the anterior edge of the mesokatepisternum; *Ae. dupreei* has no scales present in either area. The size of this species is the determining factor and should be considered when separating from abundant collections intermixed with *Ae. atlanticus*, *Ae. tormentor*, and *Ae. infirmatus* (Table 2). The scutum median scaling stripe is generally parallel and is considered wider than these other *Aedes* species, whereas the shape of these scutum scales is silvery-white and the shape of the scales are slightly curved and slender (Harrison et al. 2016).

Aedes (*Ochlerotatus*) *canadensis mathesoni* (Middlekauff, 1944).

March through April 2020, two *Ae. c. mathesoni* were observed from spring surveillance CDC light trap collections (Table 3), this observation is considered a county record for Bay County, FL. *Aedes canadensis* sister species are similar and are described with common anatomical character states: 1) the base of the wing costa entirely dark scaled, 2) the scutum covered in brown scales (Carpenter and LaCasse 1955) and 3) the scales of the palpus scattered with pale scales where the apex of the palpus is entirely scaled in white (Harrison et al. 2016). Both species have banded hind tarsomeres with apical and basal bands which are crossing the joint (Carpenter and LaCasse 1955). *Ae. c. mathesoni* has apical and basal bands on hind tarsomeres 1-2 whereas *Ae. c. canadensis* hind tarsomeres 1-4 are banded (Harrison et al. 2016). Hind tarsomere 3 on *Ae. c. mathesoni* has a very narrow basal band where the posterior of the hind tarsomere is completely scaled black and hind tarsomeres 4-5 are entirely scaled dark; hind tarsomere 5 on *Ae. c. canadensis* is completely white (Harrison et al. 2016) (Table 2).

DISCUSSION

The mission of public health mosquito control operations is to give a level of ataraxis from mosquito biting pressure, also, to protect from possible transmission of arboviruses through chemical and biological control measures. Standardized identification sub-sampling procedures are generally practiced within mosquito control operations to substantiate the abundance application thresholds for applying pesticides. This standard stands true for the typical controlling of pestiferous mosquito species such as *Ae. taenioryhnchus* (Weidemann), *Ae. sollicitans* (Walker), *Cx. nigripalpus* (Theobald), and *Ae. atlanticus*. These species have synchronous patterns of emergence after pupation and can emerge up to millions of mosquitoes at once dominating their specific habitats (Haeger et al. 1954, Navar et al. 1968, O'Meara et al. 1992). Although the diversity of mosquito species can be taken out of context as sub samples do not specifically depict what is currently in the ecological environment under traditional aliquoting of samples. Identification aliquoting at gross levels can misrepresent the true sense of species diversity geographically as some mosquito species are comparable to other species and unknown introduced invasive species in low abundance can be overlooked. The paradigm of morphological considerations between *Cx. restuans* and the *Cx. pipiens* complex is well known (Apperson 2002, Andreadis 2005) although Harrison (2016) has distinguished novel character states to further easily separate *Cx. restuans* and the *Cx. pipiens* but identification of a similar smaller invasive species such as *Cx. interrogator* can still become constrained (Shin et al. 2013) when sub sampling is utilized within public health identification processes. Preliminary morphometric studies have pointed out that these characters can be used to separate by wing measurements (92% identification rate, n=25) although this is not considered a standalone character state and other morphological characters need to be included with molecular identifications to achieve a higher rate of identification confidence

(Robison et al. 2018). In Panama City Beach specimens were unable to be correctly identified and were set aside due to their size and ambiguous anatomical characters, further inspection revealed otherwise (personal communication with G. O'Meara, November 2017), was identified (Darsie and Ward 2005), and the collection was then recorded accordingly on December 11th, 2017 (Riles and Connelly 2020).

Melanoconion species, *Cx. peccator*, and *Cx. pilosus* were most likely described as *Cx. erraticus* and overlooked in the identification process. Proper identification can be an arduous task as mechanical trap fan blades can be detrimental to morphological structures removing them altogether causing difficulty in the process of identification. Collections of *Melanoconion* mosquitoes can be grossly misrepresented when population abundance is disproportionate where *Culex pilosus* at 1.5% and *Cx. peccator* at .001% when measured against *Cx. erraticus* higher abundance in trapping collections over time in Panama City Beach. Species level of abundance indicators should be considered when the populations of *Melanoconion* in the past were overlooked due to the gross level abundances. Limited capacity for a higher degree of confidence in identifying *Melanoconion*, it is suggested by Savage and Williams (2009) to use their protocol of setting slides incorporating the female cibarial armature in conjunction with mesepimeron character states. Although incorporating this procedure would give the identifier a more conclusive identification; concerning mosquito control operations these types of slide mounts can be arduous at best and time-consuming. Identification becomes difficult to determine the numbers of scales on the mesokatepisternum and the shade of the integumental area on the mesepimeron which can be considered ambiguous by identifiers. In 2015-2016, collections of unidentifiable *Melanoconion* species were set aside (n=153). In 2017 these specimens were later sorted and identified respectively as *Cx. pilosus* and *Cx. peccator* (Darsie and Ward 2005; Burkett-Cadena 2012, Harrison et al. 2016). After correctly identifying the specimens based on morphology, females were recorded and added to the species list for Bay County (*cx. pilosus* n=129; *cx. peccator* n=24) (Riles and Connelly 2020).

Psorphora horrida natural geographic range is on the fringe of Bay County, FL and can be misidentified as *Ps. ferox* as these two "sister" species within the subgenus *Janthinsoma* can be mistaken by similar morphological character states at gross level identification (Harrison and Whitt 1996). In Bay County, *Ps. ferox* represented abundance in all the same trapping events constituted an overall 16% occurrence of *Ps. horrida* when *Ps. ferox* was present. May 2014 through November 2020, twenty-nine female *Ps. horrida* specimens have been collected, identified (Harrison et al. 2016), and cataloged in district databases (Table 2).

In 2014 what appeared be two *Mansonia* mosquitoes were collected from a state park. These specimens were extremely damaged from CDC light trap fan incursion and unable to verify by species specific character states and could only be identified to the genera by the character state of the tip of the abdomen blunt or rounded from the dorsal view where the abdominal segment VII is much wider than it is long (Carpenter and Lacasse 1955, Darsie and Ward 2005). Standard identifiable anatomical characters were displaced or no longer present making the identification process difficult toward determining species within *Mansonia*. A county record for *Ma. titllans* was recorded in early spring 2016 (n=6). 131 female mosquitoes have been collected and correctly identified (Harrison et al. 2016) up to December 2020 across 7 separate CDC trapping sites (Riles and Connelly 2020) (Table 3).

Subsampling collections for identification can cause issues for public health identifiers where the introduction of regional and/or alien invasive mosquito species especially with native invasive interactions in their deposed ecological niche. Introduced mosquito species should be in the interest of public health officials identification practice due to the unknown capacities for arbovirus transmission and specific interspecies interactions (O'Meara 1995). The past 5 years reporting

in the state of Florida has occurred on the migration and introduction of invasive species 1) *Ae. pertinax* (Shroyer et al. 2015), 2) *Cx. interrogator* (Shin et al. 2016), 3) *Cx. panacossa* (Blosser et al. 2016), 4) *Aedeomyia squamipennis* (Blosser et al. 2017), and 5) *Ae. j. japonicus* (Riles et al. 2017). Migratory mosquito species can move over county lines as depicted in the updates of mosquito species in the state of Florida (Smith et al. 2020, Connelly and Riles 2020) and since 2004 mosquito species distribution maps have not been updated in Florida. *Wyeomyia mitchellii* (Theobald) was transported from southern Florida in an exotic botanical and has become established in Escambia County, FL, 1087 kilometers from its original geographic position (Connelly and Riles 2020) indicating the movement of species within borders of Florida. Since 2014, in Panama City Beach, FL, identification of collections encompassed the whole sample of each net from each site surveyed. Subsampling protocols were not utilized where we produced a more clear and concise definition of mosquito species diversity. Identification procedures should have the capacity of detecting unknown mosquitoes especially vector species outside their geographic range, but in the mosquito control operational sense of time constraints, this feat can be arduous but not impossible. The use of national, state, regional identification dichotomous keys and current peer reviewed literature is vital to determining current species diversity in geographic areas when determining unknown mosquito species in surveillance collections concerning public health.

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ORNAMENTAL BROMELIADS OF LOCAL BOTANICAL GARDENS SERVE AS PRODUCTION SITES FOR PYRETHROID-RESISTANT *CULEX QUINQUEFASCIATUS* (SAY) IN COLLIER COUNTY, FLORIDA

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Subject Editor: Derrick Mathias

ABSTRACT

The Naples Botanical Garden, located in Collier County, Florida, attracts over 220,000 visitors each year. The gardens house a collection of plants from around the world, including a featured area for over 100 species of exotic and native bromeliads. Ornamental bromeliads have previously been investigated to define their "tank" structure as a haven for mosquito eggs and larvae. The Naples Botanical Gardens was investigated for the presence of juvenile mosquitoes inhabiting large-tanked bromeliads. A survey of mosquito species inhabiting bromeliads in the gardens indicated that the most abundant species was *Culex quinquefasciatus*. With the ongoing threat of vector borne diseases such as West Nile virus, the abundance of vector mosquitoes and heavy tourist traffic in the gardens, insecticide resistance testing was performed on *Cx. quinquefasciatus* originating in the gardens in order to assess the ability of pyrethroid-based insecticides used by the local vector control agency to successfully target this species in the event of a disease outbreak. We identified pyrethroid resistance in *Cx. quinquefasciatus* collected from Naples Botanical Gardens, and that oxidase activity was the primary mechanism responsible for its pyrethroid resistance status.

Key Words: bromeliads, botanical gardens, *Culex quinquefasciatus*, insecticide resistance, pyrethroids

INTRODUCTION

Florida has seen an increase of imported and local transmission of mosquito-borne disease in recent years. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) represent important vectors responsible for recent outbreaks of Zika virus (McAllister et al 2020) and dengue virus (Graham et al 2011, FLDOH 2020) in Florida. Furthermore, the state is endemic with several arboviruses transmitted by *Culex* mosquitoes, including West Nile virus (Blackmore et al 2001) and St. Louis encephalitis virus (Harwood et al 1979). Current disease vector control strategies rely on a combination of integrated pest management (IPM) approaches, including the application of insecticides targeting both the juvenile and adult stages. Therefore, it is crucial for vector control agencies to recognize potential disease vector habitat and identify best management practices in a time where resistance to control materials is on the rise.

Exotic and native ornamental bromeliads are a popular choice for urban residential and commercial landscaping in Florida due to their tropical esthetics and easy care. Bromeliads have previously been investigated to

distinguish them as a production site for juvenile mosquitoes due to their water-holding "tank" structure, which can serve as a haven for mosquito eggs and larvae. Recent studies in Miami-Dade County have revealed *Ae. aegypti* successfully propagates within tank-type bromeliads and are the dominant species found in ornamental bromeliads in urban environments (Wilke et al 2018, Wilke et al 2019, Wilke et al 2020). In order to assess the relationship between disease vector mosquito species and bromeliads in Collier County, Florida, the Collier Mosquito Control District began a district-wide survey of mosquito species found in bromeliads within urban residential and commercial settings, as well as high-traffic tourist destinations.

The Naples Botanical Garden located in Collier County, Florida attracts over 220,000 visitors and tourists each year. The gardens house a collection of plants from around the world and includes a featured area for over 100 species of exotic and native bromeliads. With the influx of tourists in the area, the Naples Botanical Gardens was investigated for species diversity to identify the presence of disease vector mosquitoes in their large-tanked bromeliads. Furthermore, due to the identification of pyrethroid-resistant *Ae. aegypti* (Estep et al 2018) and *Cx. quinquefasciatus* (Lucas et al 2020) in Collier County, pyrethroid-resistance status and mechanisms underlying resistance were assessed for the most abundant disease vector species in the gardens.

MATERIALS AND METHODS

Bromeliad survey. From May 2019 to October 2019 the Naples Botanical Gardens were routinely visited for operational inspections to survey for the presence of immature stages of mosquitoes in their exotic large-tank bromeliad collections. Inspection dates were subject to availability of operations personnel and attempted weekly subject to weather. Bromeliad water reservoir tanks were drained with the aid of manual plastic pumps (turkey basters). Juvenile mosquitoes were brought back to the laboratory and larvae were identified by morphology using taxonomic keys. Total number of each species collected was recorded, and sample volumes were then normalized to the volume of a standard larval dipper (350 mL) (BioQuip Products Inc., Rancho Dominguez, CA). After identifying and counting mosquito larvae, mosquitoes were brought back to the insectary regulated at 28°C, 80% relative humidity and a constant light/dark (14 h 10 h) cycle. Species richness (S) (the number of mosquito species present) was determined and abundance (P(i)) (proportion of the total number of i^{th} species) was calculated in Microsoft Excel for each collection date.

It is important to note that in August the Naples Botanical Gardens had treated their bromeliads with Merit 75WSP (AI: Imidacloprid) (Bayer Environmental Science, Cary, NC) for the Mexican Bromeliad Weevil, likely resulting in a crash of juvenile mosquito production from the bromeliads. While the gardens were still visited weekly, mosquito larvae were not detected until mid-October. Further, mosquito larvae were not identified in high enough abundance after mid-October to warrant operational inspections.

Insecticide susceptibility tests. The susceptibility to pyrethroids of *Cx. quinquefasciatus* collected from Naples Botanical Gardens was evaluated using the Centers for Disease Control and Prevention (CDC) bottle bioassay protocol (Brogdon and McAllister 1998a; Brogdon and Chan 2010; CDC 2020) as previously described (Lucas et al 2020). Adult female *Cx. quinquefasciatus* 3-5 days old were used for the assays. Three assay bottles using approximately 20-25 female mosquitoes each were exposed to the CDC diagnostic dose of the technical grade insecticides of either d-phenothrin (Sumithrin®) (22 ug/mL), pyrethrum (15 ug/mL) or naled (2.25 ug/mL) (CDC, 2017); acetone was used as a control treatment. Mosquitoes were also exposed to the formulated products, Anvil 10-10Ó (10% Sumithrin, 10% PBO) (Clarke Inc., St. Charles, IL), Merus 3.0Ó (5% pyrethrins) (Clarke Inc., St. Charles, IL) and DibromÓ Concentrate (87.4% naled) (AMVAC Chemical Corp., New Port Beach, CA). Formulated products were diluted in acetone to yield the equivalent CDC diagnostic dose of

active ingredient. Knockdown was recorded at 10 min, 15 min and then every 15 min for 2 h with the exception of Dibrom/Naled which was recorded every 15 min for 2 hr. The published CDC diagnostic time for technical grade insecticides against the susceptible *Cx. quinquefasciatus* Sebring colony was used for classification of resistance status (CDC, 2017). Diagnostic times for formulated products were not developed, and instead the corresponding times for technical grade insecticides were used as reference. Collections were classified as resistant or susceptible using the World Health Organization (WHO) guidelines (WHO 2013; CDC 2020): 98%–100% mortality at the recommended diagnostic time indicates susceptibility; 80%–97% mortality at the recommended diagnostic time suggests developing resistance, <80% mortality at the recommended diagnostic time suggests resistance. Percent mortality was determined using a modified formula from Abbott (1925) and an average was produced between the three technical replicates. Graphical analysis was produced using GraphPad Prism 8 (GraphPad Software, San Diego, CA).

Phenotypic expression of knockdown resistance (*kdr*). A variation of the CDC Bottle Bioassay can be used to determine if a target site mechanism, such as the presence of the *kdr* allele that results in an amino acid substitution in the voltage-gated sodium channel, contributes to the resistance status (CDC 2020). In this way, phenotypic expression of *kdr* resistance can be determined by evaluating a population for recovery 24 h post-treatment. Another round of bottle bioassays were performed on *Cx. quinquefasciatus* collected from the Naples Botanical Gardens to assess phenotypic expression of knockdown resistance (*kdr*) by allowing mosquitoes to recover for 24 h post-exposure according to CDC guidelines (CDC 2020) and as previously described (Lucas et al 2020). Three assay bottles using approximately 20-25 female mosquitoes each were exposed to the CDC diagnostic dose of the technical grade insecticides of either d-phenothrin (Sumithrin), pyrethrum or naled, as described above. After 2 h, all exposed mosquitoes were transferred to holding cages and provided a 20% sucrose solution. Mortality was recorded at 24 h post-exposure. Percent recovery was determined, and an average was produced between the three technical replicates as previously described (Lucas et al 2020). Graphical analysis was produced using GraphPad Prism 8.

Analysis of metabolic resistance. Similar to identifying resistance attributed to target-site-mutations, a variation of the CDC Bottle Bioassay using enzyme inhibitors can be used to determine resistance attributed to metabolic detoxification enzymes (Brogdon and McAllister 1998b; CDC 2020). In order to assess the effect of metabolic resistance in *Cx. quinquefasciatus* mosquitoes collected from Naples Botanical Gardens, synergists were used according to the protocol described by the CDC (Brogdon and McAllister 1998b; CDC 2020). Three technical replicates of approximately 20-25 mosquitoes were exposed to one of the three synergists: S.S.S-tributylphosphorotrithioate (DEF) (125 µg/bottle), which inhibits esterase activity; diethyl maleate (DEM) (80 µg/bottle), which inhibits glutathione transferase activity; and piperonyl butoxide (PBO) (400 µg/bottle), which inhibits oxidase activity. Mosquitoes were exposed to synergists for one hour, transferred to holding cages for recovery for another hour and then used in a bottle bioassay using technical grade insecticides, as described above. Graphical analysis was produced using GraphPad Prism 8.

RESULTS

Bromeliad survey. A county-wide operational survey of bromeliads was performed in Collier County to map bromeliad locations producing juvenile mosquitoes, the results of which are beyond the scope of this project and used solely for operational decision making. From May 2019 to October 2019 a total of 2267 juvenile mosquitoes were collected from large-tank bromeliads located in the Naples Botanical Garden. These collections comprised of three main genera – *Aedes* (Meigen, 1818), *Culex* (Linnaeus, 1758) and *Wyeomyia* (Theobald, 1901) (Table 1).

From these genera, five main taxonomic units were identified (Table 1; Figure 1A), including *Ae. aegypti*, *Ae. albopictus*, *Wyeomyia mitchellii* (Theobald), *Cx. nigripalpus* (Theobald) and *Cx. quinquefasciatus*. The most abundant species inhabiting bromeliads was *Cx. quinquefasciatus*, accounting for nearly 75% (CI 95% 0.728-0.764) of all specimens collected (Table 1; Figure 1B). *Cx. nigripalpus* was the second most abundant, accounting for 15% (CI 95% 0.0136-0.166) of the total specimens collected (Table 1; Figure 1B). *Ae. albopictus, Ae. aegypti* and *Wy. mitchellii* were the least abundant and comprised 5.6% (CI 95% 0.049-0.068), 0.8% (CI 95% 0.005-0.013) and 0.1% (CI 95% 0.000-0.003) of the total specimens collected, respectively (Table 1; Figure 1B). While five main taxonomic units were identified in total, species richness (S) on any given collection day consisted of 3 to 4 (Table 1; Figure 1A), with the identification of the two least abundant species identified only once (Table 1). *Cx. quinquefasciatus*, *Cx. nigripalpus* and *Ae. albopictus* were the most common species identified throughout the collections.

Insecticide susceptibility tests. With *Cx. quinquefasciatus* being the most abundant species in the gardens, the identification of pyrethroid resistance in Collier's *Cx. quinquefasciatus* mosquitoes (Lucas et al 2020), and the recent incorporation of pyrethroid-based insecticides into the Districts IPM program, we asked whether *Cx. quinquefasciatus* from Naples Botanical Gardens also harbors pyrethroid resistance. Technical grade products corresponding to the active ingredients found in formulated products used by the District were chosen for the assay: d-phenothrin (Sumithrin), pyrethrum and naled. D-phenothrin and pyrethrum (pyrethrins) are active ingredients in the pyrethroid-based insecticides, Anvil 10-10 and Merus 3.0, respectively, and have been recently incorporated into the Districts adulticide program. Naled is the active ingredient of an organophosphate-based insecticide, Dibrom Concentrate, also used by the District.

Culex quinquefasciatus from the Naples Botanical Gardens had a high level of pyrethroid resistance with an average corrected percent mortality rate of 2.78% for technical grade pyrethrum (Figure 2A) and 5.13% for Merus 3.0 (Figure 2B) at the CDC diagnostic time of 45 min. Further, average corrected percent mortality was 20.59% and 33.33% for technical grade d-phenothrin (Figure 2C) and Anvil 10-10 (Figure 2D), respectively. Mosquitoes were susceptible to the organophosphate naled, with complete mortality using both the technical grade and formulated product for naled at the CDC diagnostic time of 45 min (Figure 2E-F). Taken together these data signify that *Cx. quinquefasciatus* mosquitoes collected from ornamental bromeliads of the Naples Botanical Gardens are resistant to pyrethroid-based insecticides, but not organophosphate-based materials. The published CDC diagnostic dose for *Cx. quinquefasciatus* and CDC diagnostic times for technical grade insecticides were used as reference, as previously described (Lucas et al 2020). While using the technical grade pyrethrum CDC diagnostic time for Merus 3.0 may present a flaw in our comparisons, it is important to note that assays using Merus 3.0 displayed roughly 40% mortality even at the 2 h timepoint.

At this time, resistance status of other species identified within the gardens has not been assayed. Resistance status of *Ae. aegypti* within the District is well known (unpublished data; Estep et al 2018) and operational concerns regarding pyrethroid-resistance in *Cx. nigripalpus, Ae. albopictus* and *Wyeomyia* spp have not surfaced.

Detection of knockdown resistance. Recent studies on Collier's *Cx. quinquefasciatus* has shown presence of the *kdr* mutation, L1014F, and phenotypic expression of knockdown resistance in some parts of the county (Lucas et al 2020). We asked whether the Naples Botanical Garden *Cx. quinquefasciatus* collection harbors *kdr*-attributed resistance and if this has a significant impact on its pyrethroid resistance status. Phenotypic expression of knockdown resistance can be determined by evaluating a population for recovery 24 h post-treatment. After completion of the CDC bottle bioassay with technical-grade d-phenothrin and pyrethrum, *Cx. quinquefasciatus* were transferred

Table 1. Naples botanical gardens collections.

	Botanical Gardens Collections							
Date	*Aedes aegypti*	*Aedes albopictus*	*Wyeomyia mitchellii*	*Wyeomyia vanduzeei*	*Culex nigrapalpus*	*Culex quinquefasciatus*	Unknown/ Others	Total
5/24/2019	0	1 (0.16)	0	0	1 (0.16)	150 (24.71)	11 (1.81)	163 (26.84)
5/31/2019	0	0	0	0	37 (7.62)	401 (82.56)	29 (5.97)	467 (96.15)
6/4/2019	0	1 (0.18)	0	0	62 (11.27)	75 (13.64)	0	138 (25.09)
6/14/2019	0	32 (5.09)	0	0	127 (20.20)	104 (16.55)	0	263 (41.84)
6/28/2019	0	36 (14.82)	0	0	35 (14.41)	8 (3.29)	4 (1.65)	83 (34.18)
7/8/2019	0	18 (4.58)	0	0	1 (0.25)	49 (12.47)	10 (2.55)	78 (19.85)
7/15/2019	0	31 (7.75)	0	0	29 (7.25)	90 (22.50)	0	150 (37.50)
7/26/2019	0	3 (0.60)	2 (0.40)	0	49 (9.80)	128 (25.60)	0	182 (36.4)
7/31/2019	0	8 (2.8)	0	0	0	48 (16.80)	1 (0.35)	57 (19.95)
8/2/2019 #	0	1 (1)	0	0	0	2 (2)	3 (3)	6 (6)
10/16/2019	18 (8.4)	0	0	0	0	637 (297.27)	25 (11.67)	680 (317.33)
Total	18 (8.4)	131 (36.99)	2 (0.40)	0	341 (70.97)	1692 (517.38)	83 (26.99)	2267 (661)
Total P(i)	0.008 (0.013)	0.056 (0.056)	0.001 (0.0006)	0	0.150 (0.107)	0.746 (0.783)	0.037 (0.040)	
Total CI 95%	0.005-0.013	0.049-0.068	0.000-0.003	0.000-0.002*	0.0136-0.166	0.728-0.764	0.029-0.045	

Data is expressed as: Total number of species collected (normalized number to the volume of a standard larval dipper).
8/1/19 Treatment of bromeliads with Merit 75WSP (AI: Imidacloprid) for Metamasius callizona (Chevrolat) (Mexican Bromeliad Weevil)
* One-sided 97.5% confidence interval.

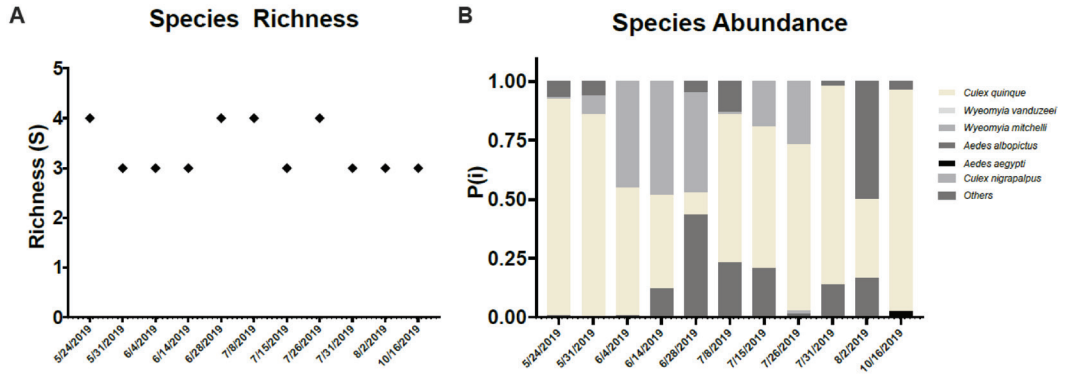

Figure 1. Bromeliad survey performed at the Naples Botanical Gardens, including species richness (A) and species abundance (B). Species richness (S) represents the number of mosquito species present. Species abundance (P(i)) represents the proportion of the total number of i^{th} species. Species richness and abundance were calculated in Microsoft Excel for each collection date.

to holding cages and assessed for recovery 24 h post-exposure. With 2 h of exposure to pyrethrum and d-phenothrin, *Cx. quinquefasciatus* collected from Naples Botanical Gardens reached a knockdown of 44.34% and 45.93% (Fig. 3A), respectively. After a 24 h recovery period, percent mortality was reduced for both technical grade materials (Fig. 3A). These results suggest that *Cx. quinquefasciatus* from Naples Botanical Garden may display phenotypic characteristics of *kdr*-associated pyrethroid resistance. Genotyping of the gardens *Cx. quinquefasciatus* collections for *kdr* mutations, such as the leucine (L) to phenylalanine (F) substitution at residue 1014 (L1014F), within the voltage-gated sodium channel gene would provide information regarding the presence or absence of these *kdr* mutations. However, phenotypic expression of *kdr* mutations through recovery assays performed in this study provides information regarding potential operational significance.

Detection of metabolic resistance. To assess the role of metabolic resistance mechanisms on the resistance status of Naples Botanical Garden *Cx. quinquefasciatus*, mosquitoes were treated with synergists to inhibit oxidase (PBO), esterase (DEF), or glutathione transferase (DEM) activity. After exposure to the synergist PBO, Naples Botanical Garden *Cx. quinquefasciatus* mosquitoes displayed a rescue of the resistant phenotype observed with exposure to pyrethrum only at the CDC diagnostic time of 45 min. Exposure of Naples Botanical Garden *Cx. quinquefasciatus* to PBO prior to CDC bottle bioassay using pyrethrum resulted in 94.12% mortality, while pyrethrum alone resulted in 2.28% mortality at diagnostic time (Fig. 3B-C). Treatment with DEF resulted in a partial reduction of the resistant phenotype with a mortality of 25.41% at diagnostic time (Fig. 3B-C). Treatment with DEM was similar to pyrethrum only, and resulted in a mortality of 3.80% at diagnostic time (Fig. 3B-C). Together these results suggest that oxidase activity plays the primary role in the pyrethroid resistance status of Naples Botanical Garden *Cx. quinquefasciatus*, while esterase activity may play partial role in resistance within this field collection.

DISCUSSION

Monitoring resistance status is essential for the selection of proper control methods to target vector species. It was recently reported that *Ae. aegypti* populations in Florida, including in Collier County, exhibit a high frequency of *kdr* mutations and increased pyrethroid resistance (Estep et al 2019). Furthermore, field collections of *Cx. quinquefasciatus* from highly urbanized areas within Collier County, have been shown to be resistant to pyrethroid-based control materials – resistance being associated with oxidase and esterase metabolic process, and

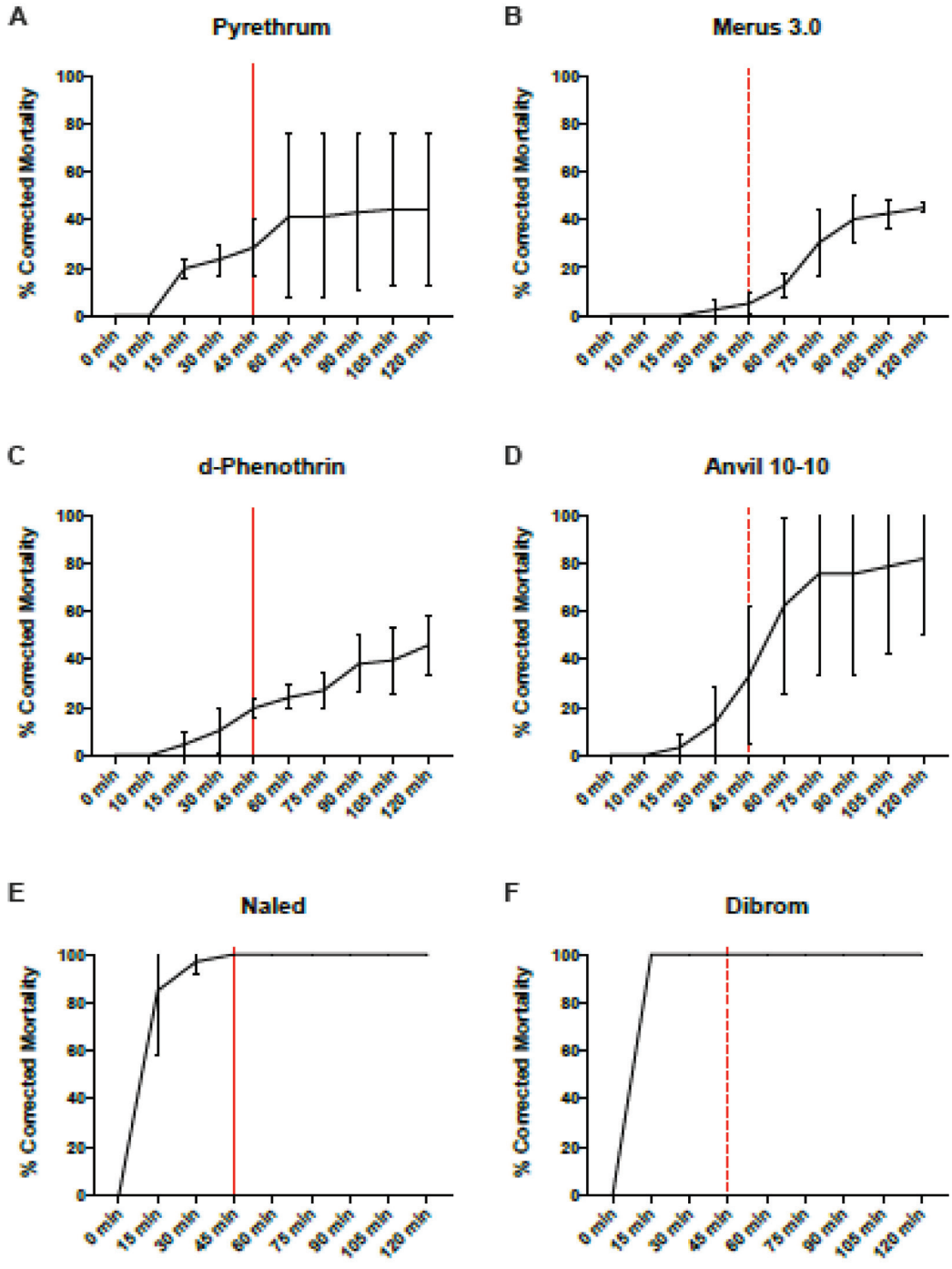

Figure 2. Centers for Disease Control and Prevention (CDC) bottle bioassays for *Cx. quinquefasciatus* collected from the Naples Botanical Gardens. CDC bottle bioassays using technical-grade insecticides: (A) 15 µg/ml pyrethrum, (C) 22 µg/ml d-phenothrin (Sumithrin®), and (E) 2.25 µg/ml naled. CDC bottle bioassays using formulated products: (B) Merus 2.0®, (D) Anvil 10-10®, and (F) Dibrom® Concentrate. Each formulated product was diluted in acetone to yield the equivalent CDC diagnostic dose of active ingredient per bottle. Solid vertical red line indicates published threshold for CDC diagnostic dose of the susceptible *Cx. quinquefasciatus* Sebring colony. Threshold times for formulated products (dashed vertical red lines) are unknown but provided for reference. Data represent 3 technical replicates and are shown as mean ± SD.

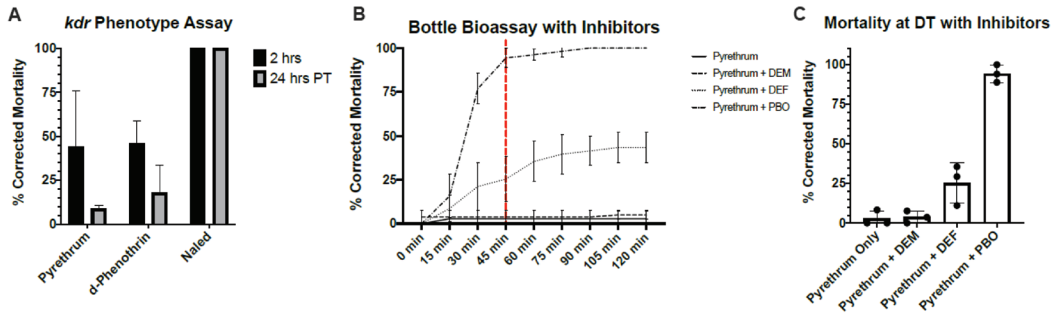

Figure 3. Identification the pyrethroid resistance mechanism including knockdown (*kdr*) and metabolic resistance. (A) Phenotypic expression of knockdown resistance in *Cx. quinquefasciatus* mosquitoes collected from the Naples Botanical Garden. Percent recovery at 2 h postexposure for Cambier Park and Sabal Palm. Data represent 3 technical replicates and are shown as mean ± SD. (B-C) Centers for Disease Control and Prevention (CDC) bottle bioassay using 15 µg/ml pyrethrum in conjunction with exposure to 1 of 3 synergists: S.S.S-tributylphosphorotrithioate (DEF) (125 µg/bottle), diethyl maleate (DEM) (80 µg/bottle), and piperonyl butoxide (PBO) (400 µg/bottle), which inhibits oxidase activity. Data represent 3 technical replicates and are shown as mean ± SD. (B) Dashed vertical red line indicates published threshold for CDC diagnostic dose of the susceptible *Cx. quinquefasciatus* Sebring colony.

kdr mutations (Lucas et al 2020). Merus 3.0, which uses 5% pyrethrins as an active ingredient, is currently being utilized in the Districts rotary-wing aircraft targeting smaller adulticiding treatment blocks. The Naples Botanical Garden often falls within these treatment blocks. With the on-going threat of vector borne disease, including West Nile virus, in Southwest Florida, it is imperative to understand the effectiveness of pyrethroid-based adulticides on vector species in these areas, and in particular, high-traffic and tourist dense locations.

Our survey for vector species inhabiting the exotic bromeliad collections of the Naples Botanical Gardens identified the presence of *Cx. quinquefasciatus, Ae. aegypti, Ae. albopictus* and *Cx. nigripalpus*. As the most abundant disease vector identified within the gardens, we asked whether our current adulticiding methods utilizing pyrethroid-based control materials would effectively reduce *Cx. quinquefasciatus* population numbers in the area. We identified pyrethroid resistance in *Cx. quinquefasciatus* collected from Naples Botanical Gardens, and that oxidase activity was the primary mechanism responsible for its pyrethroid resistance status. Furthermore, esterase activity and resistance attributed to *kdr* mutations may also play a role in Naples Botanical Gardens *Cx. quinquefasciatus* resistance status. These results are consistent with our previous studies of nearby field collections of *Cx. quinquefasciatus* from Cambier Park, an urban locality within the District (Lucas et al, 2020).

Anvil 10-10 contains the synergist PBO, and may serve as a more efficacious product against *Cx. quinquefasciatus* harboring pyrethroid resistance attributed to oxidase activity. While mortality at diagnostic time was 33.33% for Anvil 10-10, our CDC bottle bioassay results presented here indicated that Anvil 10-10 was more efficient than Merus 3.0 and d-phenothrin alone (Figure 2). Furthermore, after 2 h Anvil 10-10 had the highest mortality at just above 80% (Figure 2), suggesting that the PBO included in the formulation may play a role in its efficacy. However, if multiple resistant mechanisms are present within a mosquito population, such as esterase and *kdr*-associated resistance identified in the collections taken from the Naples Botanical Garden, then the inclusion of PBO may not be enough to rescue the pyrethroid-resistant phenotype. Vector control programs should consider multiple resistance mechanisms in relation to pyrethroid resistance identified in the field. Furthermore, bottle bioassay results may not be representative of field efficacy. If a synergized pyrethroid-based product is being considered for targeting pyrethroid resistant mosquitoes then cage trials should be performed to ensure efficacy.

Independent of wide area adulticiding practices by the local vector control agency, there are limited options to prevent vector mosquitoes from inhabiting ornamental bromeliads at the juvenile stage. Treatment using residual applications of adulticide to bromeliads have been shown to provide adequate control of *Ae. albopictus* (Bibbs et al 2018). The insect growth regulator (S)-methoprene has been successful in controlling *Ae. aegypti* production within bromeliads (Ritchie and Broadsmith 1997). Furthermore, *Bacillus thuringiensis israelensis (Bti), Bacillus sphaericus (BS)* and Spinosad-based larvicides in granular, tablet and liquid form are also readily available. All these methods require "boutique" treatments of individual production sites, which may not be feasible for the vector control program. While public outreach may assist in the reduction peridomestic container breeding species (Healy et al 2014), it is impractical to solicit the removal of ornamental bromeliads in domestic habitats or halt conservation efforts for exotic and native bromeliads by botanical gardens. As such, identifying which adulticide materials are most suitable for controlling these species originating from such habitat is essential to a well-rounded IPM program. Through a robust IPM approach, including a combination the abovementioned control measures and public outreach, vector species inhabiting bromeliads can be reduced.

ACKNOWLEDGMENTS

The authors thank the Collier Mosquito Control District (CMCD) Board of Commissioners, Executive Director Patrick Linn and all the employees at CMCD who participated in sample collection and technical assistance. We also thank the staff at the Naples Botanical Gardens, including Cameron Cole, for providing CMCD access to their facility for this study. We also thank the CDC Southeastern Center of Excellence in Vector Borne Disease for providing funding for CMCD's research internship program. Further, we thank the CDC Division of Vector-Borne Diseases for offering mosquito control districts with free Insecticide Resistance Kits and guidelines for assessing insecticide resistance and resistance mechanism evaluations.

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RELATIONSHIP OF PRECIPITATION AND HABITAT TO THE SPATIAL AND TEMPORAL ABUNDANCE OF *AEDES ATLANTICUS* AND *AEDES INFIRMATUS* IN ST. JOHNS COUNTY, FLORIDA

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Guest Editor: Mohamed F. Sallam

ABSTRACT

The purpose of this study was to perform descriptive and inferential analyses to better understand the presence of the abundant mosquito species *Aedes atlanticus* and *Aedes infirmatus* in St. Johns County, northeastern Florida. Historical surveillance data (2010-2019) obtained from Anastasia Mosquito Control District of St. Johns County, St. Augustine, FL, was organized to graph temporal mosquito abundance trends and inverse distance weighted (IDW) interpolation was used to map spatial distribution patterns of mosquitoes. Precipitation and habitat composition were investigated as spatiotemporal predictors of mosquito abundance using Pearson's correlation statistics. There were considerable and inconsistent fluctuations in the population abundance of *Ae. atlanticus* and *Ae. infirmatus* across and within individual surveillance seasons during the last decade. Precipitation was significantly associated with total county-wide mosquito population counts by season (*Ae. atlanticus,* $R = 0.810$, $p = 0.005$; *Ae. infirmatus,* $R = 0.850$, $p = 0.002$), while the association with weekly mosquito population trends was inconsistently significant across species, lag time, and years. The proportion of surrounding land covered by upland forest, water, and agriculture was associated with species abundance at the spatial level of individual trap sites. Overall, the results identify that *Ae. atlanticus* and *Ae. infirmatus* share a spatiotemporal relationship and are similarly impacted by rainfall and habitat type. Findings of the study might help to inform improved surveillance by integrating IDW estimation maps with current district resources and improved knowledge of species' ecology.

Key Words: *Aedes atlanticus*, *Aedes infirmatus*, GIS, spatial, temporal, precipitation, land cover

INTRODUCTION

Aedes atlanticus Dyar & Knab and *Aedes infirmatus* Dyar & Knab are floodwater mosquitoes that are aggressive biters and nuisance pests. There is field evidence that these mosquitoes are vectors for several arboviruses including keystone virus (KEYV) and eastern equine encephalitis virus (EEEV) (Bigler et al. 1976, Wellings et al. 1972, LeDuc et al. 1975, Roberts and Scanlon 1975). The high abundance of these two mosquito species in St. Johns County [AMCD 2017] combined with the detection of both KEYV and EEEV within county borders may pose a risk to public and veterinary health. However, the population distribution and ecological patterns of *Ae. atlanticus* and *Ae. infirmatus* in St. Johns County and the entirety of North Florida are not well described. Such knowledge could help to inform and improve surveillance and control programs in the county.

Geographic information systems (GIS) and remote sensing have emerged as powerful tools in mosquito control efforts by offering insights into geographic distribution and spatial clustering which help to understand historical patterns and overall dynamics of mosquito vector populations (Hungerford 1991) and arbovirus transmission (Sallam et al. 2016a, b). A spatiotemporal analysis can be used to determine static or dynamic hotspots of abundant mosquito populations and guide control efforts to predict and pre-

emptively manage these areas. Additionally, it is equally important to observe and study the potential ecological drivers of mosquito distributions to better understand a species' presence and geographical movement. Such knowledge can be used to optimize surveillance and control programs, especially when considering ecological drivers of vector populations as indirect risk factors for arbovirus transmission.

The objective of this study was to explore the spatiotemporal distribution of *Ae. atlanticus* and *Ae. infirmatus* and examine potential ecological drivers of mosquito abundance and distribution in St. Johns County based on the historical surveillance records archived by Anastasia Mosquito Control District (AMCD) of St. Johns County, FL.

MATERIALS AND METHODS

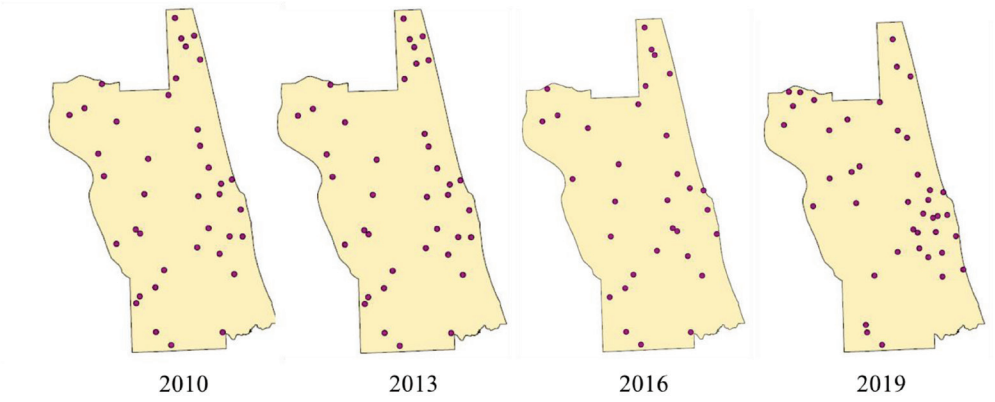
Study Area. St. Johns County is located on the northeastern part of Florida and covers 1,650 km^2 between the St. Johns River and the Atlantic Ocean coastline (Fig. 1). The region has a humid subtropical climate with an average high temperature of 90°F (32.2 °C) in the warmest month and an average low temperature of 46°F (7.8 °C) in the coldest month (Weather Atlas). The environment is characterized by a range of salt and fresh water habitats and is classified as an Eastern Florida Flatwoods Class IV ecoregion by the Environmental Protection Agency (United States Environmental Protection Agency). The population estimate for 2019 was 249,734 residents compared to an estimated 190,646 residents in 2010 (Florida Health Charts). Shapefiles of St. Johns County waterways, roads, and mosquito adulticide zones were retrieved from AMCD GIS archives.

Figure 1. Map of Florida and county borders of St. Johns County, Florida

Mosquito surveillance dataset. AMCD uses the U.S. Centers for Disease Control and Prevention (CDC) light traps (John Hock, Gainesville, FL) baited with octenol for its seasonal surveillance program which typically runs April-November every year. During 2010-2019 light traps were equipped with a plastic collection container with a pesticide stick, 12V battery, light bulb, and an octenol lure stick (synthetic semiochemical, Biosense). Traps were set out in designated field locations (one trap per site, number of sites dependent on season) once every week for approximately 24 hours. Afterwards, light traps and collections were transported to the AMCD laboratory for species identification and database recordkeeping using appropriate taxonomic keys (Darsie and Ward 2005).

Data preparation & exploratory data analysis. Historical CDC light trap surveillance data (2010-2019) were retrieved from AMCD database records. Spreadsheet data of weekly mosquito trap collections for a subset of years (2010, 2013, 2016, 2019) were reorganized, georeferenced, and compiled into total counts per week and per trap location for both *Ae. atlanticus* and *Ae. infirmatus* (Fig. 2a, b). The total sum of adult female mosquitoes collected at an individual or all CDC light trap sites over the course of a (standardized) surveillance season was used as a measure of total seasonal count. The standardization process entailed comparing the start and end dates of all surveillance seasons and tailoring a standard period that matched the shortest surveillance season (twenty-six weeks; early May-early November) to all years with approximately equivalent start/end dates. Mosquito counts that fell outside of these weeks for any year were excluded from all temporal and spatial analyses. Total seasonal

(a)



(b)

Surveillance season		*Aedes atlanticus*		*Aedes infirmatus*	
Year	No. traps	Min-Max	SD	Min-Max	SD
2010	39	0-599	100.31	0-123	28.38
2013	39	0-5,910	1,238.49	0-227	50.64
2016	32	0-4,956	1,094.25	0-73	16.2
2019	41	0-768	139.63	0-167	32.39

Figure 2. CDC light traps and spatial heterogeneity of mosquito abundance: (a) CDC light trap locations for the years 2010, 2013, 2016, and 2019. (b) Descriptive statistics of mosquito counts at 2010, 2013, 2016, and 2019 trap sites. The minimum, maximum, and standard deviation (Min, Max, SD) were calculated using the total count of mosquitoes collected at ***individual*** trap sites over each twenty-six-week season to emphasize ***spatial heterogeneity***. Averaged seasonal abundances for each species during these years are listed in Table 1.

counts were then utilized as proxy measures for assuming total seasonal abundance at either an individual location or on a county-wide scale. County-wide totals were averaged by the number of active trap sites in a given surveillance season to account for differences in number of traps deployed (Table 1). Four years of the ten-year timespan were initially singled out for spatiotemporal analyses due to a limited time capacity; however, early exploratory data analysis motivated the integration of total count data from the remaining six years of the decade in temporal analyses (not spatial) to better understand the fluctuations in county-wide trends across separate surveillance seasons.

One problem that arose was the presence of null values for weeks within the standardized season due to no trap collections that week for unknown or extreme weather-related reasons. Data imputation was used to overcome this issue by averaging the weekly county-wide abundance data between the prior week and following week surrounding a missing data point. Four years had no missing collection weeks while other years had one, two (most), or in one case three weeks missing. Other imputation techniques, such as averages for a particular collection week across the remaining ten years, did not produce valid estimates. Imputation was not used to replace missing weekly count values at individual trap sites and thus did not impact spatial analyses.

Due to observations from initial exploratory analyses, rainfall was chosen as a potential environmental predictor of temporal mosquito abundance. Daily and seasonal precipitation summaries (April 2010-November 2020) were downloaded from the Hastings 4 NE, FL US GHCND: USC00083874 weather station in St. Johns County via the

Table 1. The total and average abundance of *Ae. atlanticus* and *Ae. infirmatus* for every year 2010-2019 using the total combined count of all mosquitoes from all traps active during the twenty-six-week season. To describe the full timeline of 2010-2019, the minimum, maximum, mean, and standard deviation (Min, Max, Mean, SD) were calculated using the compiled averaged abundance of mosquitoes from ***all*** trap sites over each season (Average/trap) across all years to emphasize ***temporal heterogeneity*** among separate surveillance seasons. Use of raw collection counts; imputed values were not included in the calculations of this table.

Surveillance season		*Ae. atlanticus*				*Ae. infirmatus*			
Year(s)	No. traps	Total		Average/trap		Total		Average/trap	
2010	39	1,525		39.10		679		17.41	
2011	39	405		10.38		948		24.31	
2012	39	6,861		175.92		2,05		71.92	
2013	39	26,769		686.38		1,221		31.31	
2014	38	19,938		524.68		987		25.97	
2015	32	20,670		645.94		322		10.06	
2016	32	26,337		823.03		452		14.13	
2017	32	47,088		1,471.50		9,935		310.47	
2018	32	15,098		471.81		1,175		36.72	
2019	41	3,236		78.93		589		14.37	
		Min	Max	Mean	SD	Min	Max	Mean	SD
2010-2019		10.38	1471.5	490.29	457.54	10.06	310.47	55.75	91.25

National Oceanic and Atmospheric Administration Climate Data Online Search tool (https://www.ncdc.noaa.gov/cdo-web/search).

Spatial analysis. GIS Esri ArcMap 10.7.1 software was used to create maps and perform all spatial functions and calculations. Total seasonal mosquito trap counts were linked to the county shapefile with XY coordinates (latitude, longitude) of sampling sites. All data sets were projected to the (GCS_NAD_1983_2011) geographic coordinate system and the Albers Equal Conical Area projection coordinate system (NAD_1983_2011_Florida_GDL_Albers). Portions of the base county shapefile were erased with the overlay of the waterways to clarify the boundaries of the county landmass and adulticide zones during creation of interpolation maps.

Previous entomological and vector studies have employed interpolation techniques to estimate mosquito species abundance at non-sampled locations, particularly using the inverse distance weighted (IDW) method (Allen and Shellito 2008, Cleckner et al. 2011, Sarfarz et al. 2012, Sumaye et al. 2012, Suganthi et al. 2015, Dunphy et al. 2019, Saffawati et al. 2019). IDW was chosen for these analyses due to its low processing power, comprehensiveness, and simplified interpolation that does not necessitate the more sophisticated math parameters of other methods. All interpolation for mosquito abundance was performed using ArcMap 10.7.1 default settings (variable distance, twelve minimum neighbors) due to the uneven and extensive spread of sampling sites. The default decay power of two was also kept because this is within the standard range of environmental interpolation studies (literature cited above). A biologically relevant fixed distance (i.e. mosquito flight range) was not possible to include in the input parameters because the distance between most sampling sites exceeded such a distance threshold. IDW maps were created for each singled-out year (2010, 2013, 2016, 2019) or an aggregate total across the four years using georeferenced seasonal totals. For several statistical analyses, the layer attributes of estimated mosquito counts were extracted from IDW maps for 2010, 2013, and 2016 by setting the input location points as the CDC light trap sites from the 2019 surveillance season, which had the highest number of active trap sites (Fig. 2 a,b). This allowed comparison of (estimated) mosquito abundance across the timeline from locations where a trap was not permanently placed throughout the four years.

Habitat composition analysis. This methodology was based on studies by Moncayo (2000) and Kelen et al (2012). Land use/land cover (LULC) data sourced from the 2014 St. Johns River Water Management District (SJRWMD) LULC dataset which was downloaded from the Florida Geographic Data Library (https://www.fgdl.org/metadataexplorer/explorer.jsp). St. Johns River Water Management District provides a localized and finely detailed classification of LULC with a maximum one hundred LULC codes to describe polygon land plots. Digital orthophotography and classification of St. Johns County was accomplished by SJRWMD in 2015. There are four levels of LULC classifications defined by the SJRWMD with Level 1 being the broadest and Level 4 as the most specific. Level 2 classification codes were originally chosen but were later adjusted to resemble Level 1 codes with seven aggregated categories; (1) residential and built-up, (2) agriculture, (3) upland non-forested, (4) upland forested, (5) water, (6) wetlands, and (7) transportation, utilities, and barren.

Buffer zones were drawn around all thirty-two mosquito trapping sites from the 2016 surveillance season. Data from other surveillance years (2010, 2013, and 2019) were not included in these analyses to avoid confounding of potentially significant LULC change between years, e.g. urbanization. The buffer radii were 2.2 km or 1.4 km to account for the published flight range of *Ae. atlanticus* and *Ae. infirmatus,* respectively (Morris et al. 1991, Verdonschot and Besse-Lototskaya 2014). The values of the total area of Level 2 LULC codes within each buffer zone were extracted to calculate the proportions of LULC classes within the total buffer area surrounding individual CDC light trap sites. The identity tool was used for retrieval of the exact LULC classification of any individual polygon cell that contained a mosquito trap site.

Statistical analysis: For non-spatial analyses, classical Pearson's correlation was used to test the relationships between two quantitative variables (total seasonal precipitation vs total seasonal county-wide mosquito abundance, total weekly precipitation vs total weekly county-wide mosquito abundance). Precipitation was lagged at two and three-week intervals for correlation tests with mosquito counts to account for the timespan needed for mosquito development from egg to adult. Spatially-referenced mosquito abundance at individual trap sites was represented by total seasonal counts. For habitat composition analyses and related Pearson's correlations, abundances at individual 2016 trap sites were log(n+1) transformed to achieve a more normalized distribution (Williams 1937, Bidlingmayer 1969). Mosquito abundance was separately compared against values of percent buffer coverage by each LULC class. Non-parametric statistics (Kruskal-Wallis) were used for any testing of quantitative variables between categorical groups (surveillance season) due to the non-normal distribution of all datasets (O'Hara and Kotze 2010). All statistical tests were performed using SPSS Statistic 26 software and were species-specific.

RESULTS

Spatiotemporal patterns. Temporal: Mosquito abundance data (2010-2019) were compared by year, month, and week with measurements of average number mosquitoes per trap to account for the varied number and location of light traps across the ten years. Overall, abundance of *Aedes atlanticus* was higher than that of *Ae. infirmatus* in CDC light trap collections every year except for 2011 which had an abnormal pattern due to most traps having comparatively low or zero counts of *Ae. atlanticus* throughout the duration of the surveillance season**.** Both species demonstrated variation in total mosquito abundance across individual surveillance seasons; however, abundance trends (population rise or decline) over the decade were similar between the two species, aside from 2012-2013 and 2015-2016 when total seasonal counts of *Ae. atlanticus* and *Ae. infirmatus* contrastingly increased or decreased in number compared to the previous year (Table 1). There was a positive correlation ($R = 0.674$, $p = 0.046$) between the average (standardized) seasonal abundance between the

Table 2. The Kruskal-Wallis non-parametric method was used to test for significant differences in the mean seasonal mosquito abundance between all the years 2010, 2013, 2016, and 2019 (input values as seasonal aggregates of mosquitoes collected at individual CDC light traps). Tukey's test for post hoc analysis allowed multiple pair-wise comparisons (2010 vs 2013, 2013 vs 2019, etc.) to resolve which pairs of years had statistically significantly differences. The bolded test statistics (χ^2, p-value) represent statistical significance between samples (n = df + 1).

	Ae. atlanticus			*Ae. infirmatus*		
Years compared	χ^2	p-value	df	χ^2	p-value	df
2010 - 2019	**51.482**	**<0.001**	3	**10.748**	**0.013**	3
2010 vs 2013	**-55.603**	**<0.001**	1	-21.949	0.158	1
2010 vs 2016	**-59.427**	**<0.001**	1	-3.346	1.000	1
2010 vs 2019	-14.285	0.864	1	-9.325	1.000	1
2013 vs 2016	-3.825	1.000	1	-18.603	0.444	1
2013 vs 2019	**-41.318**	**<0.001**	1	**-31.273**	**0.008**	1
2016 vs 2019	**-25.143**	**<0.001**	1	-12.671	1.000	1

two species. *Aedes atlanticus* often had peak activity in the latter half of the season with a gradual increase in emergence until the end of October. In contrast, average peak abundance for *Ae. infirmatus* typically occurred June-August and then remained low for the rest of the season. There were also week-to-week fluctuations which were not constant between years as the population growth curves of both species were highly variable over the standardized twenty-six-week season for 2010-2019 with differences in magnitude and seasonal timing of population peaks between years. *Aedes infirmatus* populations generally emerged before *Ae. atlanticus* according to collection data from weeks preceding the standardized timeline. Both species reached one to several dramatic population peaks during May-November and most of these crests lasted one to two weeks until there was a noticeable decrease in the number of mosquitoes collected.

Spatial: The variation in seasonal trap collections across different traps sites

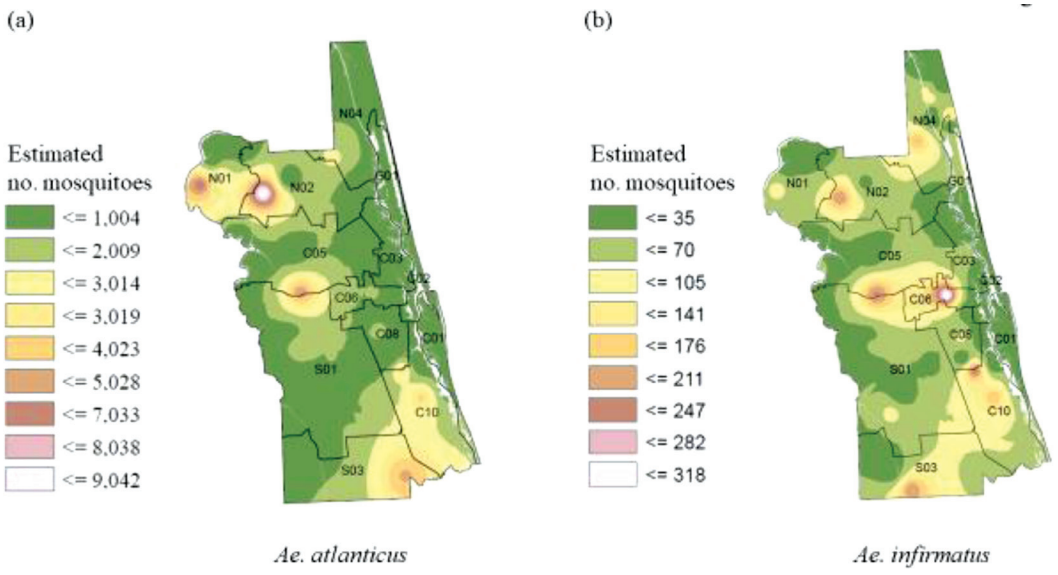


Figure 3. Aggregated IDW surfaces with overlay of adulticide zones: The aggregates of total seasonal counts of (a) *Ae. atlanticus* and (b) *Ae. infirmatus*, collected at all trap site locations of 2010, 2013, 2016, and 2019, were used to create an IDW surface for each species. The symbology classification was set as equal intervals and includes both the minimum and maximum values of estimated number of mosquitoes (i.e. mosquito abundance) across the county. A shapefile of AMCD's 2020 adulticide route zones was overlaid the IDW raster surfaces to outline zones with historical hotspots of mosquito populations.

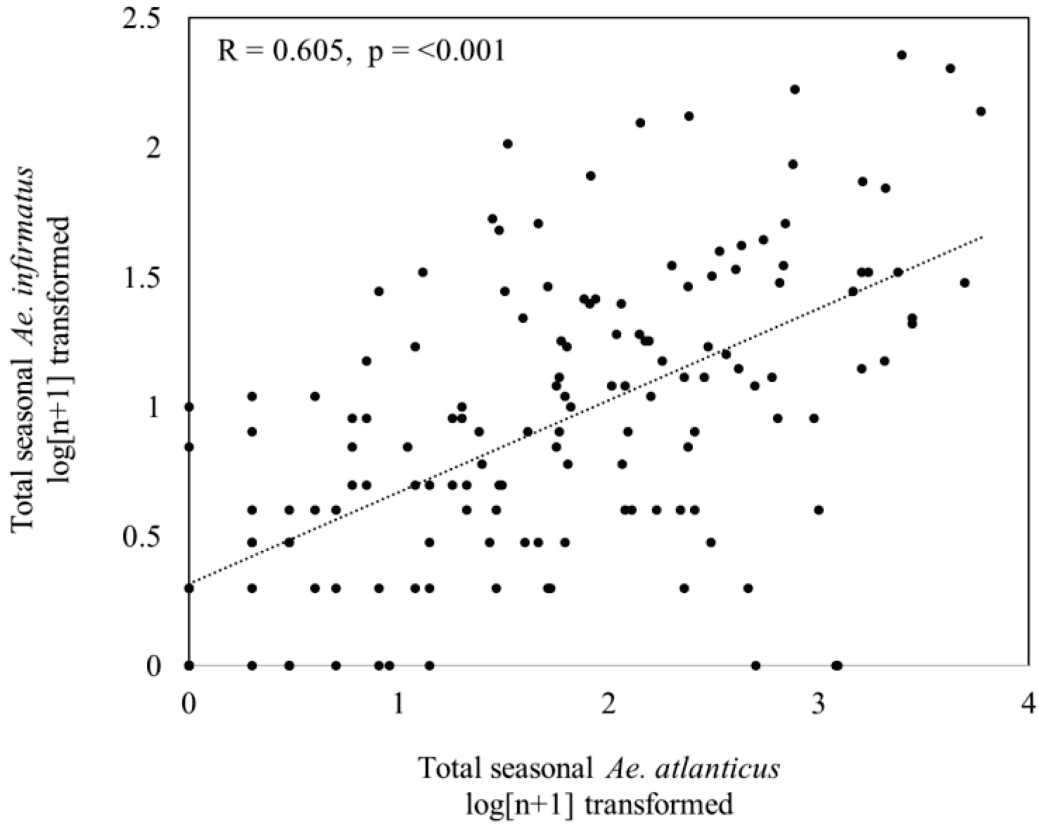


Figure 4. Spatial association of mosquito species at CDC light trap sites: Scatterplot of *Ae. atlanticus* versus *Ae. infirmatus* mosquito abundance counts (log[n+1] transformed) at individual CDC light trap locations for all traps from 2010, 2013, 2016, and 2019. If a trap location was used for more than one year, each year's entry and mosquito count were counted as a new matched pair and were graphed as a separate scatter point. Results for Pearson's test for linear correlation located on plot.

(Fig. 2b) indicated a high level of spatial and temporal heterogeneity of mosquito abundance throughout St. Johns County. Total mosquito counts for each trap site for the years 2010, 2013, 2016, and 2019 were compared (Table 2). Both species had a statistically significant difference in mean seasonal abundance across the four years. For *Ae. atlanticus*, a Tukey post hoc test showed this difference was due to significant differences in the distributions of mosquito abundance counts between either peripheral year (2010, 2019) versus the middle two years (2013, 2016). There was no significant difference in county-wide abundance with 2010 vs 2019 or 2013 vs 2016. For *Ae. infirmatus*, the significance of the Kruskal-Wallis test was driven by a significant difference only between years 2013 vs 2019.

IDW surface maps created with data from individual surveillance seasons showed shifting clusters of mosquito population hotspots with inconsistent intensities across the four years (data not shown). Standardization of ArcGIS classification symbology for a single species clarified the differences in expected population abundances and distributions from year to year. The maps showed evidence of differences in county-wide mosquito abundance between years; however, did not show evidence of any chronologically consistent shifts in mosquito distribution over the decade. Thus, an aggregate of seasonal counts across all four years was used to create a hot spot map that defined three broad historical clusters of *Ae. atlanticus* and *Ae. infirmatus* (Fig. 3). An overlay of adulticide zones, used by the dis-

trict to delineate areas for adulticide fogging missions, provides a map for areas to target control of these two species. Overall, the key clusters of these mosquitoes seem to overlap with noticeable differences in spread and intensity. Although the hotspots of these two species may contrast within a single year (data not shown), the aggregated maps show an overall association of the spatial presence of these species. Pearson's correlations did demonstrate a moderate positive correlation (Fig. 4) between the total seasonal abundance of *Ae. atlanticus* and *Ae. infirmatus* collected from the same site when compiled from all four years and across individual years (Table 3). Correlations with estimated abundance values that were extracted from locations of 2019 trap sites from the appropriate IDW surfaces yielded similar strengths of spatial correlation (Table 3). These linear trends indicate that the presence and population growth trends of *Ae. atlanticus* and *Ae. infirmatus* were likely influenced by similar factors within the same geographic location.

Ecological drivers. Precipitation: One indicator of a potential driver of mosquito populations was the occurrence of large spikes in collection counts that were preceded by extreme weather events, e.g. hurricanes. Total seasonal rainfall was significantly correlated to average county-wide mosquito abundance (per all traps in one season) for *Ae. atlanticus* (R = 0.810, p = 0.005) and *Ae. infirmatus* (R = 0.850, p = 0.002) (Fig. 5a,b). Total weekly rainfall was inconsistently correlated to total mosquito abundance per week at both two and three-week lags in rainfall (Table 4). Outlier data points noticed in scatterplots were for the most part due to a week of heavy rainfall that also happened to be a peak rainfall week for the season. These outliers often drove the significant correlation and when removed from the data set the significance disappeared. This event happened most frequently with *Ae. atlanticus*. In fact, seasonal precipitation peaks were often followed by major seasonal *Ae. atlanticus* population peaks. This was not as often the case for *Ae. infirmatus* which often experienced population peaks long before or long after the onset of major precipitation events. All significant correlations were positive, aside from tests with 2018 *Ae. infirmatus* when removal of a precipitation outlier resulted in both a newly significant negative coefficient of determination at a three-week lag (R = -0.407, p = 0.043) and a switch from a significant positive to a significant negative correlation at a three-week lag (R= -0.437, p = 0.029).

Habitat composition: Population abundance of *Ae. atlanticus* at 2016 CDC light trap locations had a strong positive linear correlation to the percentage of buffer area filled by upland forests (R = 0.806, p = <0.001) and a strong negative correlation to the percent area covered by the LULC class of water (R = -0.704, p = <0.001) (Fig. 6). Population abundance of *Ae. infirmatus* also had positive and negative correlations to percent upland forest (R =0.406, p = 0.021) and percent water (R = -0.385, p = 0.029), respectively (Fig. 7). *Aedes infirmatus* had an additional negative correlation to percent agriculture in the buffer zone (R = -0.428, p = 0.015). The four other LULC classes did not share any significant association with either species.

Table 3. Positive spatial association of mosquito species: Pearson's correlation was used to find best-fit line between the total *Ae. atlanticus* and total *Ae. infirmatus* collected at the same trapping location across all years (2010-2019) or within an individual season (2010, 2013, 2016, and 2019) (**observed**). The same tests were performed using data extractions from IDW maps made with collection records of individual surveillance seasons (**IDW estimates**). These estimated abundance values were extracted using the coordinates of all forty-one locations of 2019 CDC light traps.

Observed		
Year(s)	R	p-value
2010	0.674	<0.001
2013	0.626	<0.001
2016	0.496	<0.001
2019	0.781	<0.001
2010-2019	0.605	<0.001

IDW estimates		
Year(s)	R	p-value
2010	0.599	<0.001
2013	0.671	<0.001
2016	0.539	<0.001
2019	0.781	<0.001
2010-2019	0.597	<0.001

DISCUSSION

This study explored the spatial and temporal patterns of two poorly understood mosquito species under surveillance by AMCD mosquito control operations. It was not surprising that precipitation was found to have an impact on seasonal and weekly population abundance trends (Weaver and Xue 2015, Weaver et al. 2013, 2020). Also, the land classes of upland forest, water, and agriculture were associated with the abundance of one or both species collected at CDC light trap sites.

Aedes atlanticus and *Ae. infirmatus* appear to share a close spatial association without a matched temporal association. There was a clear impact of precipitation on the emergence and population growth curves of both species, but *Ae. atlanticus* did seem to experience a more direct impact from weekly rainfall. It is possible that the seasonal fluctuations of *Ae. infirmatus* are partially related to the emergence patterns of *Ae. atlanticus*. If *Ae. atlanticus* is naturally more abundant it is then more likely to exceed the action threshold for fogging missions and both populations will be reduced even if *Ae. infirmatus* was not at a problematic level in the first place. This idea applies to the presence of other nuisance and vector species collected by any of the surveillance trap types used by mosquito control programs. Furthermore, there is simply a lack of sufficient literature that

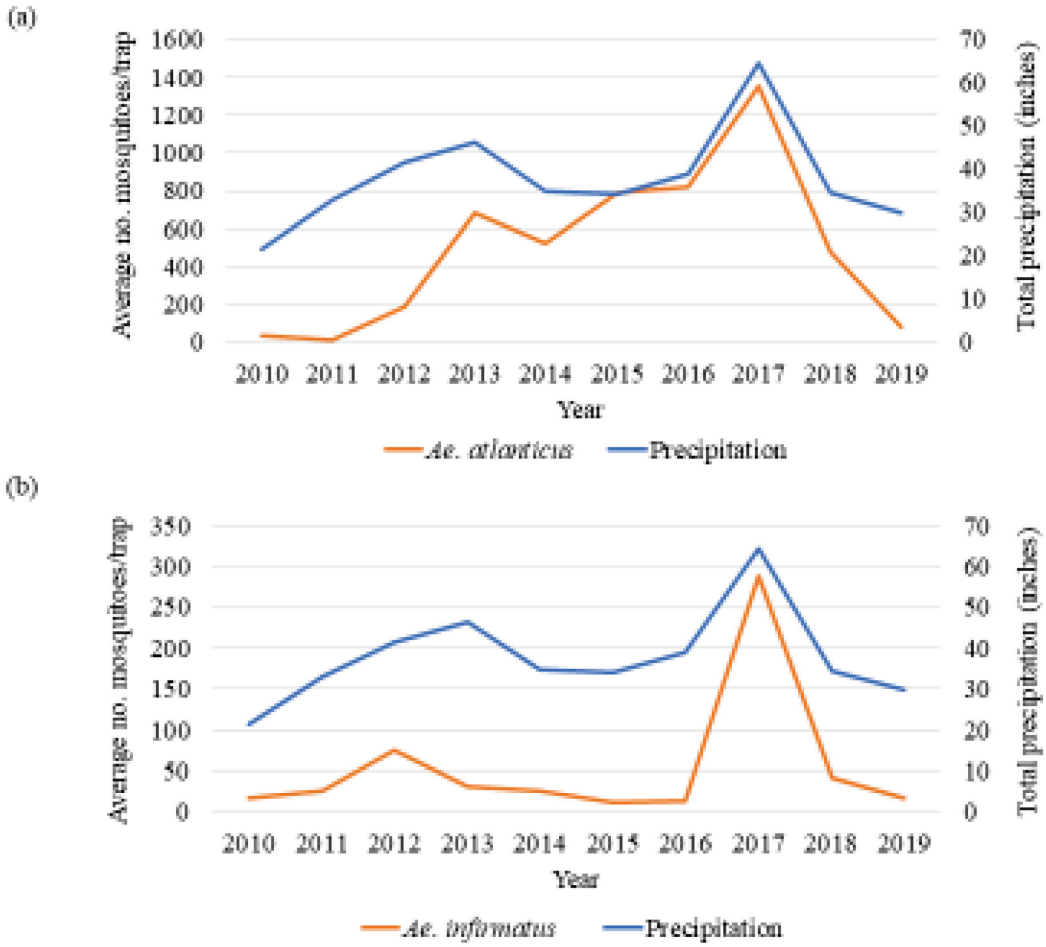


Figure 5. Seasonal mosquito abundance and precipitation relationship: A line graph depicting the total seasonal precipitation and the total seasonal abundance counts of *Ae. atlanticus* (top) and *Ae. infirmatus* (bottom) averaged by number of traps sites used in an individual year, across all years 2010-2019.

Table 4. The results of all Pearson's correlation with comparisons of total weekly precipitation and total abundance counts for a given collection week.Weekly precipitation was matched to weekly abundance counts at two- and three-week lags. All coefficients and p-value are listed, and significant correlations are bolded. * = removing outlier(s) made p-value insignificant, ** = removing outlier(s) made p-value significant, *** = removing outlier(s) made correlation change direction.

	Ae. atlanticus				*Ae. infirmatus*			
Precipitation	*2-week lag*		*3-week lag*		*2-week lag*		*3-week lag*	
Year	R	p-value	R	p-value	R	p-value	R	p-value
2010	**0.734**	**<0.001**	-0.056	0.785	0.226	0.267	-0.183	0.371
2011	0.114	0.580	0.342	0.087	0.153	0.455	0.336	0.940**
2012	0.229	0.261	0.255	0.209	0.152	0.458	**0.445**	**0.019***
2013	-0.209	0.305	**0.663**	**<0.001***	-0.072	0.727	-0.066	0.749
2014	0.233	0.252	**0.404**	**0.040**	0.043	0.836	0.259	0.201
2015	**0.504**	**0.009**	0.096	0.640	0.237	0.255	0.176	0.400
2016	**0.492**	**0.130**	-0.097	0.637	0.034	0.868	-0.121	0.556
2017	0.347	0.082	-0.050	0.809	0.177	0.386**	0.074	0.719
2018	**0.796**	**<0.001***	-0.040	0.847	**0.627**	**0.001*****	-0.223	0.274**
2019	**0.409**	**0.038**	0.058	0.780	0.213	0.296	0.207	0.310

compares the biology of these mosquitoes and most taxonomic references simply state that these two species are "associated" with one another. However, it is likely that there are significant biological and ecological differences which have yet to be investigated and would help explain the mismatches in temporal emergence and the disproportionate population abundance trends.

Previous studies have successfully utilized measurements of the life-cycle stages of eggs, larvae, or most commonly, adults, to create informative IDW models of mosquito distribution (Allen and Shellito 2008, Eleckner et al. 2011, Sarfarz et al. 2012, Sumaye et al. 2010, Suganthi et al. 2015, Dunphy et al. 2019, Saffawati et al. 2019, Kahamba et al. 2020) which lends flexibility to some of the resource limitations that may inhibit a regional mosquito control district. A major strength of spatial maps is that they can be understandable to key stakeholders and are applicable to real-time mosquito control operations, especially in relation to management of vector-borne disease (Eisen and Lorenzo-Fuentes 2009, Eisen and Eisen 2011). Unfortunately, few papers describe a specific implementation of mosquito control strategies based on IDW findings rather than simply promoting a generalized concept of potential applications. Sumaye et al (2012) created IDW surfaces with adult mosquito collections to directly aid development of a model for determining optimal deployment of mosquito control interventions (e.g. lure-and-kill odor baited stations). Meanwhile, Regis et al (2013) used kernel density estimation (akin to interpolated hotspot maps) of mosquito egg abundance to help evaluate a pilot evaluation of a proposed integrated control strategy. Their spatial maps identified priority areas for control efforts, communicated findings to field workers, and aided analysis of the ongoing impact on mosquito populations by the integrated control activities. The current IDW mapping protocol developed with AMCD mosquito trap records does present real-time implications for this district's field operations by allowing more targeted intervention strategies on a week-to-week basis that optimizes the capacity of a limited field technician staff. However, a realistic form of implementation would need to be considered in terms of the operational, administrative, and regulatory systems in place at the district.

Directly compared to this study's objectives, other publications have both utilized IDW to conduct spatiotemporal analyses and have also demonstrated the impact of climatic and LULC variables on mosquito distribution (Suganthi et al. 2011, Sarfaz et al. 2012). The IDW maps of *Ae. atlanticus* and *Ae. infirmatus* here demonstrated the spatial

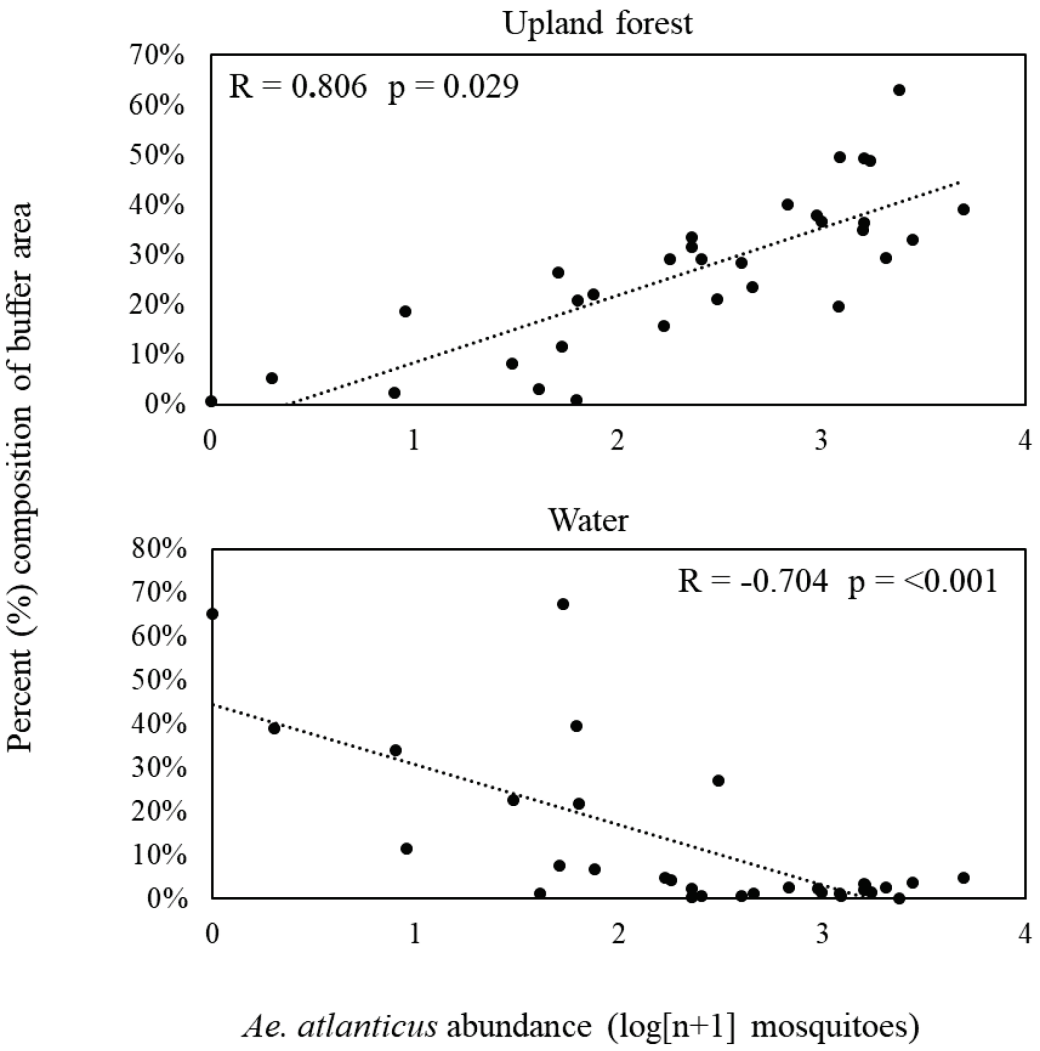


Figure 6. Habitat composition: *Aedes atlanticus:* Scatter plots with best-fit lines displaying the association between seasonal abundance (log(n+1) transformed) of *Aedes atlanticus* at a 2016 CDC light trap site and percent composition of the buffer area covered by a land cover class.

heterogeneity of species abundance in St. Johns County while habitat composition analyses then clarified likely drivers of this spatial variation, for example the result that CDC light traps sites surrounded by upland forest had collected significantly more *Ae. atlanticus* and *Ae. infirmatus*. The simplified LULC class 'Upland Forest' in this report includes the subset class coniferous forest, upland hardwood forest, mixed, and tree plantations. Tree plantations are the predominate Level 2 LULC class for St. Johns County (data not shown) and it is worthwhile to further examine this relationship to upland forest further because of the likely county-wide risk of mosquito emergence and distribution. The negative correlation with water is rational because this LULC class was an umbrella class and included lakes, reservoirs, bays, estuaries, streams, waterways, enclosed saltwater ponds, major springs, and slough waters. These are considered moving water or permanent water bodies, none of which are indicated to be the preferred ecological niche of *Ae. atlanticus* or *Ae. infirmatus* (Burkett-Cadena 2013). The negative association with agriculture with only *Ae. infirmatus* is another curiosity. One notable

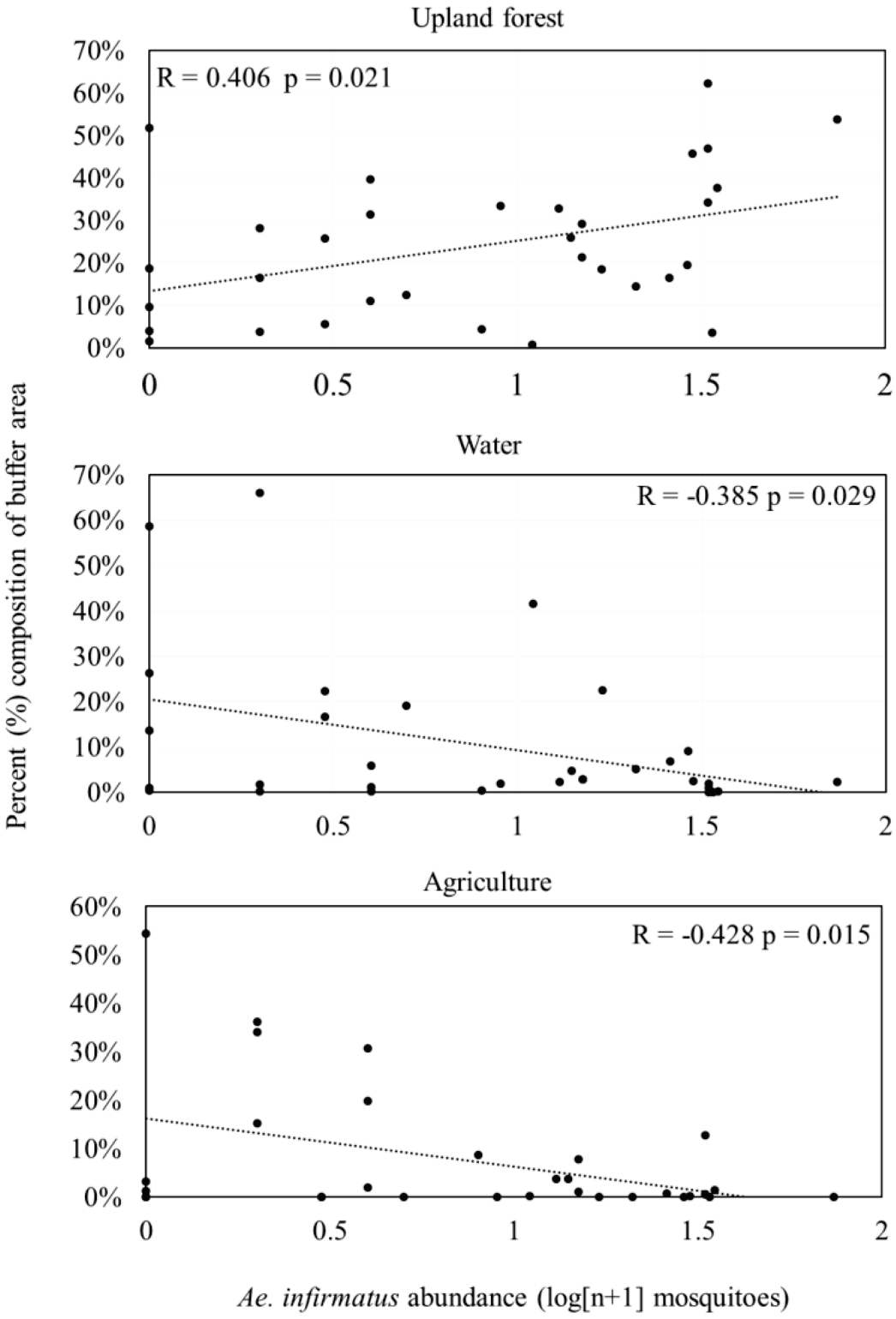


Figure 7. Habitat composition: *Aedes infirmatus:* Scatter plots with best-fit lines displaying the association between seasonal abundance (log(n+1) transformed) of *Aedes infirmatus* at a 2016 CDC light trap site and percent composition of the buffer area covered by a land cover class.

limitation of all habitat composition tests is that there was lack of consideration of the percent of other classes in a buffer which is a potential statistical issue since the proportion of a LULC class is inherently affected by the proportion of others.

This project compiled and utilized publicly available databases and developed protocols to manipulate historical mosquito control datasets. In addition, there is a large volume of additional historical data that was left out of this project, including AMCD surveillance reports from 2004-2009. One important research direction is to analyze the impact and effectiveness of direct mosquito control pesticide application efforts throughout St. Johns County. This type of evaluation is lacking in the literature and could add real value to programs with fewer resources and less capacity. Also, the observed relationship of mosquito collections to precipitation implicates the usefulness for a spatial study that specifically tracks and compares the historical emergence locations of nuisance and vector species after extreme historical weather events such as hurricanes. Recommendations for future projects are to develop stronger statistical models using multiple linear regression to better represent the complex hierarchy of climate, environment, and mosquito species dynamics more accurately. Trends in spatial distribution might be best described over a longer time than 2010-2019 or be better represented with spatial maps for continuous years. The last consideration involves developing a pragmatic integration of spatial density maps into real-time control operations and stakeholder participation. It is necessary for any interested program to account for the feasible quantity and geographic spread of light traps and to focus on reaching sufficient coverage and reliability in the areas with the greatest density of vulnerable residents.

ACKNOWLEDGEMENTS

We thank J.R. Weaver and M.K. Gaines for their background knowledge of AMCD operations and provision of access to AMCD historical data archives. We also thank L. Mao, J. Aufmuth and D. Maxwell from the University of Florida for all their guidance and feedback concerning data manipulations, ArcGIS software, and statistics. There are no conflicts of interest to report. This research was supported/funded by the Department of the Army, U.S. Army Contracting Command, Aberdeen Proving Ground, Natick Contracting Division, Ft. Detrick, MD under Deployed War Fighter Protection (DWFP) Program W911QY2010004 awarded to AMCD.

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EVALUATION OF DYNATRAP® DT160 AS AN INEXPENSIVE ALTERNATIVE TO CDC TRAPS FOR ADULT MOSQUITO MONITORING IN MALI, WEST AFRICA

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Guest Editor: Christopher S. Bibbs

ABSTRACT

Mosquito monitoring traps (i.e., CDC light traps) are crucial tools for basic vector ecology research, risk assessment, and vector control programs. Unfortunately, they are expensive which is often an issue in projects conducted in developing countries. Therefore, it would be desirable to have reliable but inexpensive alternatives based on existing consumer products. We compared an off-the-shelf DynaTrap (model DT160, CCFL tube 365 ± 3 nm UV) modified to fit a CDC trap collection bag and to use a 12V power supply, with two commonly used CDC traps: CDC Miniature Light Trap Model 512 (incandescent light, 6 Volt) and CDC Miniature Downdraft Blacklight (UV) Trap Model 912 (4-Watt blue-black-light tube, 12 Volt), in different ecological settings in southwest (Kenieroba) and northwest (Nioro du Sahel) Mali, West Africa. In northwest Mali, the modified DynaTrap caught a mean of 20.67 ± 2.8 females and 5.38 ± 1.0 male *Aedes aegypti* which was 16.55% and 10.78% more, respectively, than the CDC incandescent trap (control). The DynaTrap caught a mean of 29.75 ± 2.8 female and 17.92 ± 3.5 male *Culex quinquefasciatus*. which was 47.76% and 20.70% more than the control CDC incandescent trap. The DynaTrap caught a mean of 2.46 ± 0.5 females and 1.63 ± 0.6 males and 10.16% and 2.45% more female and male *An. gambiae s.l.*, respectively, than the CDC incandescent trap. Trap and catch means were lower at the southwest Mali site. However, trap catch proportions by sex were similar to those in the northwest. The modified DynaTrap outperformed both CDC monitoring traps for less than one third of the cost including the cost of the DynaTrap modifications.

Key Words: Surveillance traps, trap costs, *Aedes aegypti*, *Anopheles gambiae s.l.*, *Culex quinquefasciatus*, Mali

INTRODUCTION

Mosquito surveillance is a critical component of mosquito and vector management operations around the world providing important information on the population dynamics of target species present in a specific geographic area, especially those of medical, veterinary, and public health importance (Kline et al. 2006). Dacko et al. (2020) define mosquito surveillance as a systematic, rigorous, and continued effort to monitor mosquito populations over time to obtain information about distribution, abundance, and species composition. These data are used to assess the risk of mosquito-borne pathogens that cause disease outbreaks and the need for or efficacy of intervention efforts. Silver (2008) reviews the available tools to conduct this surveillance. Mosquito light traps are one of the most common tools used. In the United States, the Centers for Disease Control and Prevention (CDC) miniature light trap has been the standard light trap (Sudia and Chamberlain 1962) used by mosquito abatement districts for decades because it is portable, easy to set up, and captures a wide variety of mosquitoes. However, the expense can become prohibitive to wider scale experiments. When choosing an appropriate trap for use in developing nations one must consider cost of mosquito surveillance operations as well as trap efficacy and portability. Thus, improved surveillance strategies in these developing countries should demonstrate high levels of efficacy, field robustness, affordability, and scalability.

Vector surveillance traps are an essential tool for mosquito and vector control operations around the world. Ovitraps and gravid traps, as well as adult traps, are used to get a complete picture of the species that are found in each area (Service 1993). Adult traps typically attract mosquitoes with light or a combination of light and carbon dioxide (Kline 1994). The standard, lightweight and easy to deploy, CDC incandescent light trap has not evolved much since it's development from the late 1950s to the early 1960s by Dan Sudia, Roy Chamberlain and the CDC Equipment Development Shop (CDC, 2015). What has changed over time is the cost of these traps. Current costs in the United States for a standard CDC incandescent light trap is approximately $100 USD, plus the cost of a collection net. If an ultraviolet (UV) light source is preferred, the cost of the traps is closer to $200 USD. These prices are reasonable if only a few traps are required for a project. However, with many surveillance projects and/or control programs, many surveillance traps are needed. These costs, in bulk, can become an extreme burden on program budgets. For these reasons, we examined a lower cost UV light trap, the DynaTrap DT160, that was developed as a budget retail-use trap for consumers that could be modified easily by the manufacturer to connect to a 12-v battery. We evaluated the DynaTrap with the CDC-incandescent (model 512) and the CDC-UV (model 912) traps to determine the comparative efficacy for catching 3 commonly encountered genera of mosquitoes (*Aedes*, *Anopheles* and *Culex*) in Mali. Approximately three DynaTrap consumer traps can be purchased for the cost of one CDC-UV light trap.

MATERIALS AND METHODS

Study sites. The two sites in Mali, West Africa, chosen to study the modified DynaTrap, were Nioro du Sahel, NW Mali (-9.60475788800° N, 15.22491160900° W) and Kenieroba, SW Mali (-8.32928630400° N, 12.11465570600° W). At each site, two traps of each kind were operated over 12 consecutive nights (or day/night). Traps were rotated daily to avoid positional bias. The trials were conducted twice resulting in a total of 24 trapping days per tested trap.

Traps. The traps compared in this study were: the DynaTrap model DT160, light source: CCFL tube 365 ± 3 nm UV (Woodstream Corp., Melbourne FL, USA), the CDC incandescent model 512, light source: light bulb, incandescent (John W. Hock, Gainesville FL, USA), and the CDC-UV Trap model 912, light source: UV 4-Watt blue-black-light tube, 12 Volt (John W. Hock, Gainesville FL, USA). Typically, for consumer use, the DT160 comes equipped with the standard

120 V AC to 12 V DC power adapter. For testing purposes, the 120V power adapter was replaced with battery clips to allow the DynaTrap to directly connect to a 12 V battery source, as shown (Fig. 1a).

To increase the capacity of the DynaTrap catch container, the floor of the trap collection basket was removed, and the opening was fitted with a large (44 cm length x 35 cm lower diameter) net catch bag modified from the John Hock model 512 collection net (part number 1.42). All traps were suspended 1.5-m above the ground, either on tripods or other suitable constructions, outdoors in direct proximity to houses (Fig. 1b), with a distance of 25 m (minimum) between them. Traps were designed or modified for use with 12-v, 10-amp batteries and were spaced at least 30-m apart. After each overnight trapping period, batteries were changed, and traps were rotated sequentially between the three trap locations at each trapping site.

Mosquito species. The two nuisance mosquitoes, *Culex quinquefasciatus* and *Aedes aegypti* are invasive species and are distributed almost globally. In the USA, both species are common from early summer to autumn, while in Mali, West Africa, the same species are abundant year-round. The trials conducted in West Africa were done in a similar environment as found in the southern USA. *Anopheles gambiae s.l.*, an important malaria vector in Africa, is a representative of the genus. Others can also be found in the United States: *An. quadrimaculatus* (Eastern USA) and *An. freeborni* (Western USA).

Statistics. The numbers of mosquitoes caught within each site (male and female) were analyzed using two-way ANOVA followed by a Tukey's post-hoc test to rank significance levels. Differences were said to be significant at $P < 0.05$. Analysis was conducted using GraphPad Prism 8.00 for windows (GraphPad Software, La Jolla California, USA). The difference between the mean number of mosquitoes (± SEM), and *P*-values of the comparisons are reported in the tables 1-3.

RESULTS

As expected, all traps caught significantly more females than males regardless of site. Figures 2, 3 and 4 show the mean daily/nightly catches of mosquitoes at the two trapping sites in Mali: Nioro du Sahel and Kenieroba. In general, the DynaTrap and

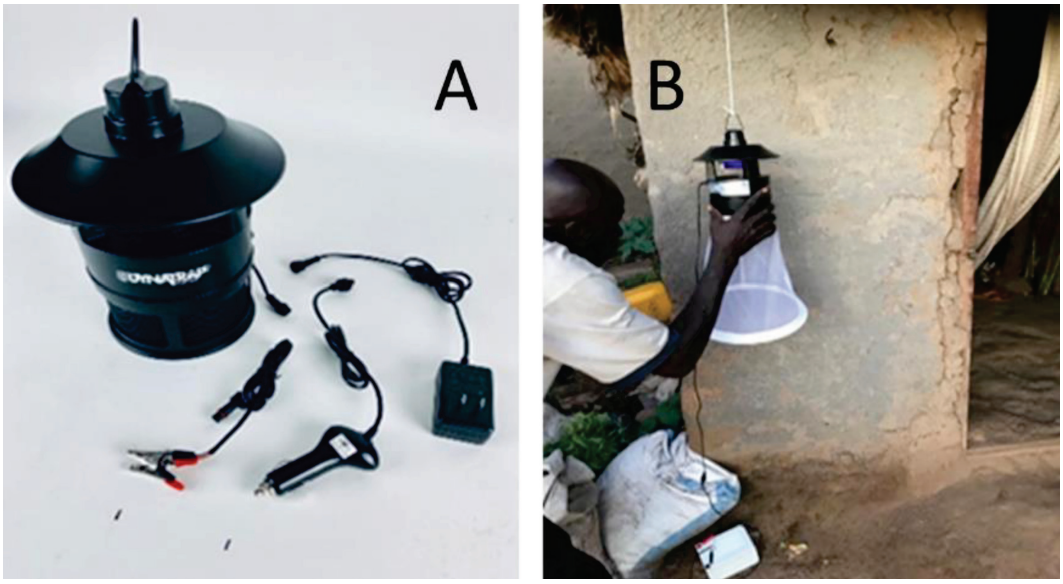

Figure 1. a) DynaTrap DT160 showing battery clips and power cord. b) DynaTrap DT160 showing modified collection net and power supply.

Table 1. Comparison of mean trap catches (by sex), within each site, of *Aedes aegypti* mosquitoes in Kenierobia and Nioro du Sahel, Mali.

Village	Sex	Trap type comparison	Mean # Trap 1 (±SEM)	Mean # Trap 2 (±SEM)	Adjusted P Value
Nioro du Sahel	Females	DynaTrap vs. CDC Trap (UV)	20.7 ± 2.8	13.3 ± 1.4	<0.001
		DynaTrap vs. CDC Trap (incandescent)	20.7 ± 2.8	3.4 ± 0.7	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	13.3 ± 1.4	3.4 ± 0.7	<0.001
	Males	DynaTrap vs. CDC Trap (UV)	5.4 ± 1.0	2.5 ± 0.5	<0.001
		DynaTrap vs. CDC Trap (incandescent)	5.4 ± 1.0	0.6 ± 0.2	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	2.5 ± 0.5	0.6 ± 0.2	<0.001
Kenieroba	Females	DynaTrap vs. CDC Trap (UV)	1.63 ± 0.4	1.0 ± 0.3	0.1
		DynaTrap vs. CDC Trap (incandescent)	1.63 ± 0.4	0.4 ± 0.2	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	1.04 ± 0.3	0.4 ± 0.2	0.056
	Males	DynaTrap vs. CDC Trap (UV)	0.4 ± 0.2	0.2 ± 0.1	0.823
		DynaTrap vs. CDC Trap (incandescent)	0.4 ± 0.2	0.1 ± 0.1	0.546
		CDC Trap (UV) vs. CDC Trap (incandescent)	0.2 ± 0.1	0.1 ± 0.1	0.892

Table 2. Comparison of mean trap catches (by sex), within each site, of *An. gambiae s.l.* mosquitoes in Kenierobia and Nioro du Sahel, Mali.

Village	Sex	Trap type comparison	Mean # Trap 1 (±SEM)	Mean # Trap 2 (±SEM)	Adjusted P Value
Nioro du Sahel	Females	DynaTrap vs. CDC Trap (UV)	2.5 ± 0.5	1.4 ± 0.5	0.012
		DynaTrap vs. CDC Trap (incandescent)	2.5 ± 0.5	0.3 ± 0.1	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	1.4 ± 0.5	0.3 ± 0.1	0.008
	Males	DynaTrap vs. CDC Trap (UV)	1.6 ± 0.6	0.3 ± 0.2	0.008
		DynaTrap vs. CDC Trap (incandescent)	1.6 ± 0.6	0.1 ± 0.02	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	0.3 ± 0.02	0.1 ± 0.02	0.841
Kenieroba	Females	DynaTrap vs. CDC Trap (UV)	22.2 ± 3.1	10.9 ± 2.1	<0.001
		DynaTrap vs. CDC Trap (incandescent)	22.2 ± 3.1	2.9 ± 0.8	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	10.9 ± 2.1	2.9 ± 0.8	<0.001
	Males	DynaTrap vs. CDC Trap (UV)	10.5 ± 2.0	4.5 ± 1.0	<0.001
		DynaTrap vs. CDC Trap (incandescent)	10.5 ± 2.0	0.8 ± 0.3	<0.001
		CDC Trap (UV) vs. CDC Trap (incandescent)	4.5 ± 1.0	0.8 ± 0.3	<0.001

Table 3. Comparison of mean trap catches (by sex), within each site, of *Culex quinquefaciatus* mosquitoes in Kenierobia and Nioro du Sahel, Mali.

Village	Sex	Trap type comparison	Mean # Trap 1 (±SEM)	Mean # Trap 2 (±SEM)	Adjusted P Value
Nioro du Sahel	Females	DynaTrap vs. CDC Trap (UV)	29.75 ± 2.8	34.5 ± 4.8	<0.0001
		DynaTrap vs. CDC Trap (incandescent)	29.75 ± 2.8	14.21 ± 2.1	<0.0001
		CDC Trap (UV) vs. CDC Trap (incandescent)	34.5 ± 4.8	14.21 ± 2.1	<0.0001
	Males	DynaTrap vs. CDC Trap (UV)	17.92 ± 3.3	14.08 ± 3.5	<0.0001
		DynaTrap vs. CDC Trap (incandescent)	17.92 ± 3.5	3.71 ± 0.8	<0.0001
		CDC Trap (UV) vs. CDC Trap (incandescent)	14.08 ± 3.5	3.71 ± 0.8	<0.0001
Kenieroba	Females	DynaTrap vs. CDC Trap (UV)	13.92 ± 2.0	11.96 ± 1.8	0.0138
		DynaTrap vs. CDC Trap (incandescent)	13.92 ± 2.0	5.04 ± 1.3	<0.0001
		CDC Trap (UV) vs. CDC Trap (incandescent)	11.96 ± 1.8	5.04 ± 1.3	<0.0001
	Males	DynaTrap vs. CDC Trap (UV)	6.46 ± 1.0	5.17 ± 1.1	0.1518
		DynaTrap vs. CDC Trap (incandescent)	6.46 ± 1.0	1.38 ± 0.5	<0.0001
		CDC Trap (UV) vs. CDC Trap (incandescent)	5.17 ± 1.1	1.38 ± 0.5	<0.0001

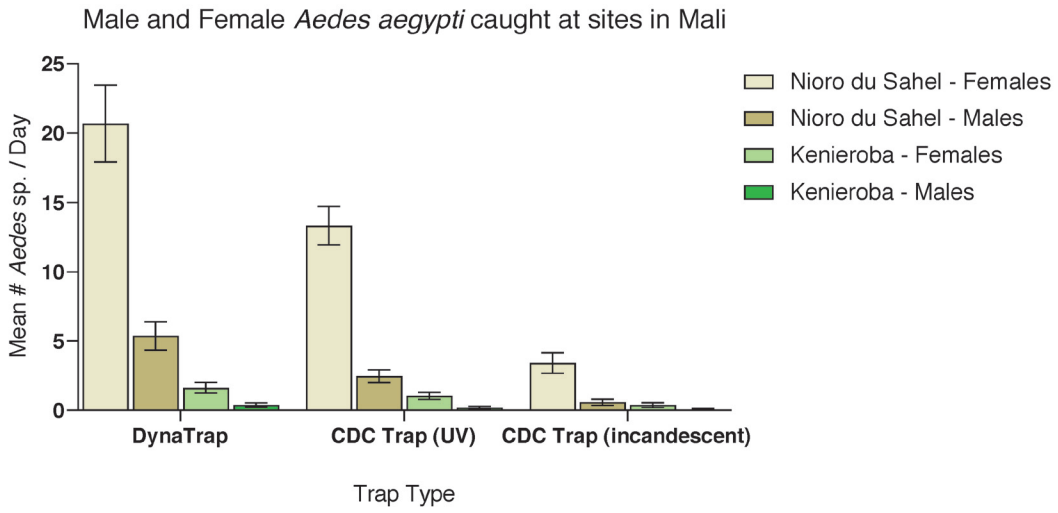

Figure 2. Average catch of male and female *Aedes aegypti.* mosquitoes per day/night ± SEM at the two trapping sites in Mali.

CDC-UV traps caught more mosquitoes than the CDC incandescent trap. In Nioro du Sahel the CDC-UV trap captured more *Cx. quinquefasciatus* females than the modified DynaTrap (Fig. 4). All of the traps caught very few males.

DISCUSSION

The World Health Organization has emphasized the need to strengthen and integrate surveillance into a major core component of strategies to combat mosquito-borne diseases (WHO 2017, 2019a). For this to happen, affected countries require inexpensive, scalable tools for monitoring, plus a set of simplified surveillance indicators. Smith et al. (2007) and WHO (2019b) have indicated that surveillance for malaria and other vector-bone diseases plays a major role in: tracking transmission; assessing susceptibility of vectors to interventions; measuring receptivity in specific locations; and predicting disease outbreaks. A number of traps are

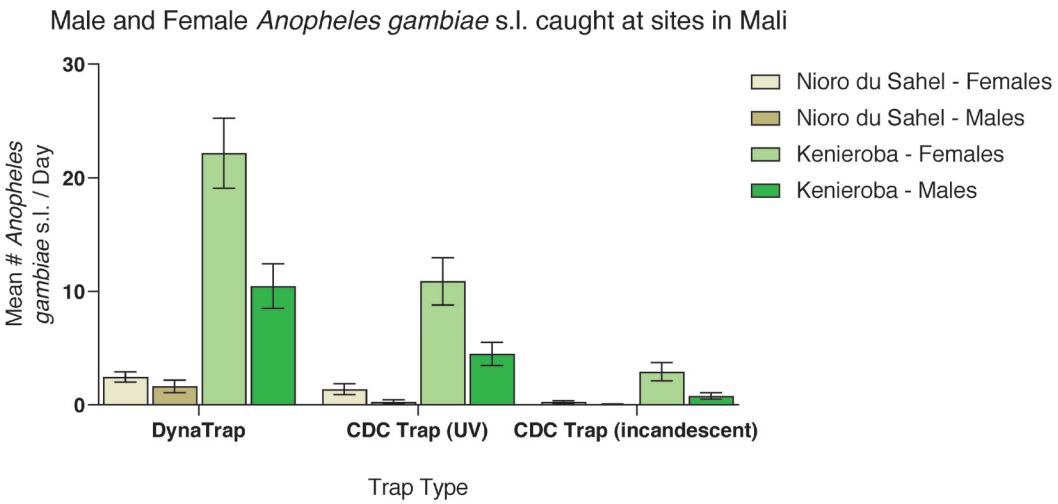

Figure 3. Average catch of male and female *Anopheles gambiae s.l.* mosquitoes per day/night ± SEM at the two trapping sites in Mali.

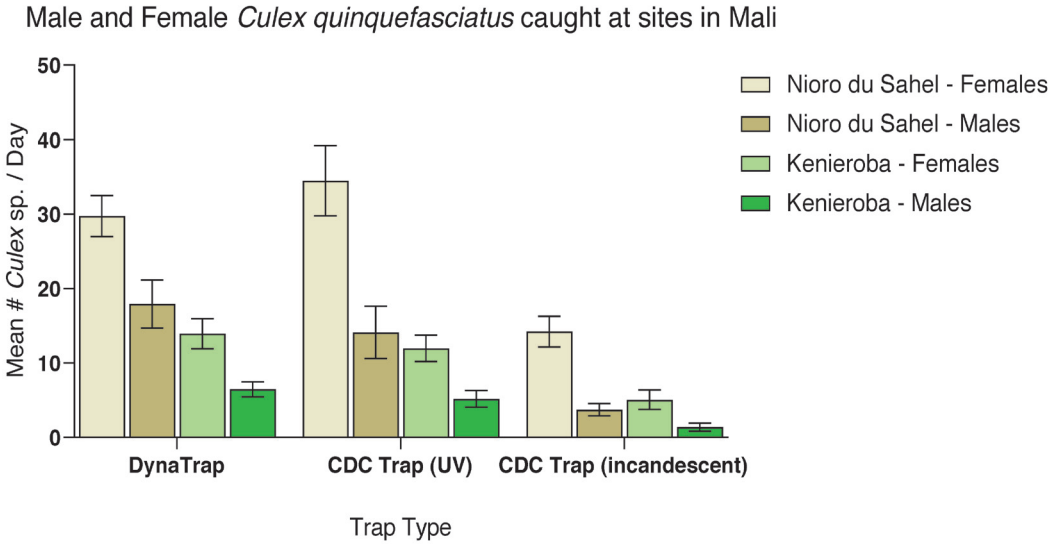

Figure 4. Average catch of male and female *Cx. quinquefasciatus* mosquitoes per day/night ± SEM at the two trapping sites in Mali.

In both ecological settings, the modified DynaTrap caught numerically but not always significantly more mosquitoes overall than the CDC incandescent trap and the CDC-UV trap. Only in Nioro du Sahel (in the case of female *Cx. quinquefasciatus quinquefaciatus*) did the CDC-UV trap catch significantly more females than the modified DynaTrap (Table 2).

on the market that have been included in operational mosquito sampling and surveillance programs (Mboera 2006, Davis et al 1995, Silver and Service 2008). Some traps have been found to be efficacious enough to be intervention devices in mosquito control systems (Rapley et al. 2009, Day and Sjogren 1994, Okumu et al. 2010). A major and commonly seen problem with many traps is poor scalability, most often because of their physical structure and relatively high cost.

The Center for Disease Prevention and Control light trap (CDC-light trap), improved by Sudia & Chamberlain (1962) by adding an incandescent light, is widely used for indoor collections of host-seeking mosquitoes (Mboera et al. 1998, Zaim and Ershadi 1986). The CDC-light trap uses light bulbs (incandescent and later, UV), battery cells, and a motor-driven fan, all of which make it expensive and difficult to maintain in many settings. Despite these challenges, the CDC-light trap is still considered one of the simplest trapping techniques, requiring only light as an attractant.

To improve surveillance strategies against vector-borne infections, new trapping devices are required that demonstrate high levels of efficacy, field robustness, affordability, and scalability. Mwanga et al. (2019) evaluated the efficacy of a new ultraviolet LED trap (Mosclean) against standard CDC incandescent light in rural south-eastern Tanzania. When simultaneously placed inside the same semi-field chamber, the Mosclean trap caught twice as many *Anopheles arabiensis* as the CDC-light trap. These traps also caught equal numbers of *An. arabiensis* and twice as many *Cx. quinquefasciatus* mosquitoes as CDC-light traps in the field. The Mosclean trap emits optimized high efficiency UV LEDs (wavelength of 365 nm) to attract mosquitoes. An additional advantage is that the lamp can run for more than 10,000 hours and therefore requires less frequent replacements than the incandescent lamps that, used in the CDC-light trap, typically run for 1200 hours or less (Viribright 2019).

In the current study, the light source of the tested trap (DynaTrap DT160) was a Cold Cathode Fluorescent Light (CCFL) tube. The trap was compared to the "gold-standard" CDC-light traps with incandes-

cent and UV light bulbs, which are commonly used for trapping mosquitoes inside and outside of human dwellings. The DynaTrap DT160 3.5 ± 10 W CCFL light source produces a wavelength of 365 ± 5 nm, drawing 0.4 A/hour from a 12V battery. In comparison, per the manufacturer's website (John Hock 2019), the Model 912, CDC style, downdraft blacklight trap uses a "4-Watt blue-blacklight tube and a very efficient transistorized inverter-ballast to provide radiation in the near-UV range (ca. 320-420 nm)". The Model 912 draws 0.5A/hour from a 12V battery. While very similar in output, better performance of the DynaTrap could be explained by the differences in the type of UV source, the narrower range of the UV spectrum, as well as the configuration of the bulb.

It is worth noting the differences in the trap catch of each species based on the site it originated from. Kenieroba, in SW Mali, has much higher levels of *An. gambiae s.l.* owing to its wetter climate and concentrated presence of human beings. Nioro du Sahel, in NW Mali, is located in an arid semi-desert has more suitable habitat for *Aedes aegypti* and *Cx. quinquefasciatus*. Although each trap type reflected this, the DynaTrap caught the most females of all species which is the goal from a disease surveillance perspective.

This study demonstrates that it is feasible to outfit commercial mosquito traps to suit the needs of field researchers which continue to be scalability, robustness, cost, and efficacy. With minimal and simple modifications, the DynaTrap DT160 was effective under field conditions where electricity may not be readily available and did so in a cost-effective manner. It also caught similar numbers of female mosquitoes of all three species evaluated and can, therefore, be used in place of the more costly CDC light traps for monitoring adult mosquitoes in the field. The DynaTrap also caught *An. gambiae s.l.* females in comparable numbers to the CDC-UV trap and can thus be good candidates for surveillance in between traps and West Africa. Future work should focus on testing this hypothesis in other regions of Africa towards a broader range of species of medical importance, a limitation of the current study.

ACKNOWLEDGEMENTS

The authors wish to thank communities in Bamako and Kenieroba, Mali that participated in the study. The trials were in part supported by the ICEMR Vector supplement Grant Number 5U19AI089672.

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PERFORMANCE OF THE ATRAKTA™ MOSQUITO LURE IN COMBINATION WITH DYNATRAP® (MODELS DT160 AND DT700) AND A CDC TRAP (MODEL 512)

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Subject Editor: Yong-Xing Jiang

ABSTRACT

The performance of the three-part mosquito lure ATRAKTA (1-octen-3-ol, ammonium bicarbonate, and lactic acid) was evaluated in two DynaTrap commercial mosquito traps (models DT160 and DT700) as well as in one model of CDC trap (model 512). Lures were evaluated fresh from the factory, after being aged in functioning traps under field conditions, and after prolonged storage in the packaging (aged for 30 days aged in functioning traps before being tested in the DynaTrap models; and two years stored in the packaging before being tested in CDC traps). The primary study questions were whether the addition of lures would increase efficacy of various trap types and whether lures would retain effectiveness after a lengthy stay on the shelf or in traps. To do this, traps with no lures, new lures and old lures were used to trap three mosquito species (*Aedes albopictus*, *Culex quinquefasciatus*, and *Anopheles gambiae*) in the field in West Africa Mali, the first two species are also common North American nuisance mosquitoes. The addition of ATRAKTA lures aged 30 days to both DynaTrap® models, and ATRAKTA lures aged two years in the packaging to the CDC trap significantly increased catches of female *Cx. quinquefasciatus* and *Ae. albopictus* mosquitoes. Aged lures did not significantly lose their attraction in comparison to lures fresh from the factory. The addition of lures to traps resulted in slight increases in catches of *An. gambiae*, but these were not statistically significant. No effect of any lures on males was observed.

Key Words: Atrakta, attractant, Dynatrap, CDC trap, *Anopheles gambiae*, *Aedes, albopictus*, *Culex quinquefasciatus*

INTRODUCTION

The success of Integrated Vector Management (IVM) programs is generally measured using surveillance traps which can be costly. In a previous study, (Traore et al., 2021), we demonstrated that the DynaTrap (Model DT160) did just as well or better at trapping *Aedes albopictus*, *Culex quinquefasciatus*, and *Anopheles gambiae* s.l. as more

costly CDC trap models. We hypothesized that the success of these surveillance traps at catching female mosquitoes could be significantly increased with a good lure. Common attractants in mosquito surveillance traps include light, host-mimicking $C0_2$, and an array of volatile compounds that emanate from plants or fungi, such as octenol (Kline, 1994), or from human skin, such as L-lactic Acid and Ammonia (Acree et al., 1968; Kline et al., 1990; Geier et al., 1999; Hoel et al., 2007). The Atrakta pod lure (Woodstream Corp., Lititz, PA, USA) is a combination of octenol (1-octen-3-ol), L-lactic acid and ammonia. All three of these compounds have been identified as mosquito attractants separately and/or in various combinations, to varying degrees of attractiveness, depending on combinations as well as mosquito species. The goal of the triple combination is to have a broader range of attraction than the individual compounds alone.

The effect of the ATRAKTA pod lures in traps was evaluated against *Cx. quinquefasciatus, Ae. albopictus* and *An. gambiae.* These first two are nuisance mosquito species in North America but are also important disease vectors there and in other countries, as well (Bhattacharya et al. 2016; Gratz 2004). They are essentially cosmopolitan in their distribution (Farajollahi et al., 2011; Kraemer et al., 2015). *An. gambiae* s.l. is an important malaria vector in Africa (Rosenthal et al. 2019). The main questions were how much the addition of the 3-part ATRAKTA pod lure would increase trap catches of these three species and if a fresh and a 30-day old lure would perform equally well in a DynaTrap model DT160 or DT700. Model 512 CDC traps equipped with either a fresh or a 2-year-old package-aged lure served as a standard trap.

MATERIALS AND METHODS

Study sites. Trials with *Cx. quinquefasciatus* were conducted in suburban Bamako (-7.89551508800° N 12.65701558800° W) on a quiet residential road bordered on both sides with drainage ditches. The traps were set up in a row, along 1 of the ditches, suspended 1.5 m above the ground from tripods, positioned between the ditch and the fences/walls of the nearby properties. The traps were 1 to 2 m from the ditch and 25 m apart.

Trials with *Ae. albopictus* were conducted in downtown Bamako (-7.92503622500° N 12.65316964500° W) on public parkland along the River Niger. The traps were set up in a row, along the river, suspended 1.5 m above the ground from tripods, positioned between herbaceous plants shaded by large trees. The traps were 20 to 30 m away from the river and 25 m apart.

Trials with *An. gambiae* s.l. were conducted in Kenieroba (-8.32928630400° N 12.11465570600° W) 60 km SW of Bamako on the flood plain of the River Niger in naturally irrigated rice fields. The traps were set up on tripods 1.5 m above the ground parallel to an irrigation ditch in which *An. gambiae* s.l. were breeding. Traps were placed 25 m apart.

Traps and lures

The following traps were used in the study: DynaTrap model DT160, light source: Cold Cathode Fluorescent Light UV (Woodstream Corp., Melbourne FL, USA); DynaTrap model DT700, light source: LED UV (Woodstream Corp., Melbourne FL, USA); CDC Trap model 512, light source, incandescent light bulb (John W. Hock, Gainesville FL, USA). ATRAKTA pod mosquito lures (Lactic Acid – 63.69%, 1-octen-3-ol – 73.36%, Ammonium; Bicarbonate – 100%; Woodstream Corp., Melbourne FL, USA; Fig. 1), both fresh from the factory and aged for 30 days in the field (12 hr per night) in functioning model DT160 DynaTrap traps before testing began, were used in the two DynaTrap models; ATRAKTA pod mosquito lures both fresh from the factory and aged two years in storage (off the shelf) were used in the model 512 CDC trap.

Trial Design. In total, nine trials, two at the same time for *Culex quinquefasciatus* and for *Aedes albopictus* but in two different habitats, and the one for *Anopheles gambiae*, were conducted during 2020. Trial I with DynaTrap model DT160 was carried out in early to

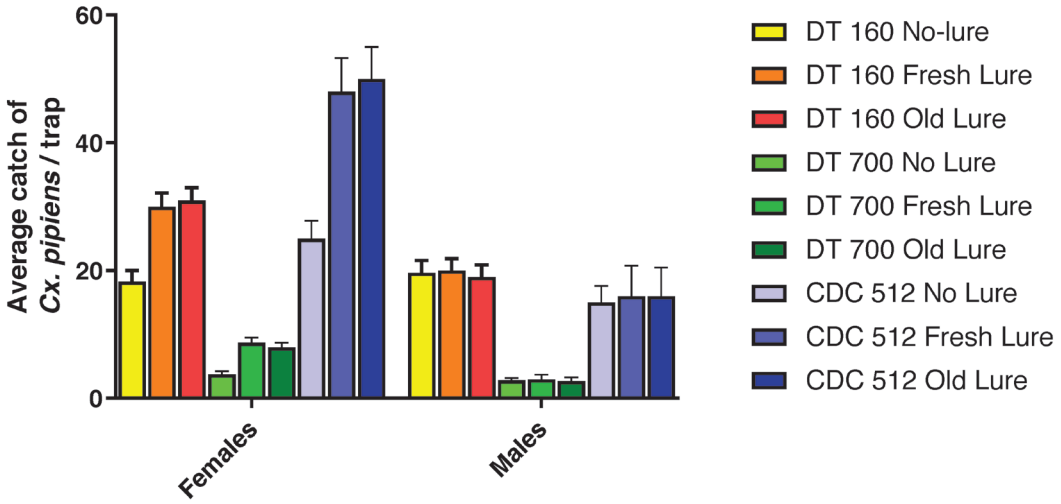

Fig. 1. Effect of the ATRAKTA pod lure on DynaTrap DT160, DT700 and CDC model 512 traps.

mid February over 14 consecutive days and nights. Trial II with DynaTrap model DT700 was carried out during mid to end of February over 10 consecutive days and nights. Trial III with CDC trap model 512, was carried out during early to mid June over 10 consecutive days and nights. Trials with *An. gambiae* s.l. were carried out in mid to late October; Trial I with DynaTrap model DT160 lasted 14 consecutive nights, Trial II with DynaTrap model DT700 lasted 10 consecutive nights and Trial III with CDC trap model 512 lasted 10 consecutive nights.

During each trial, six traps of the same kind were operated simultaneously. Two traps each were operated without lures (controls), two traps with fresh lures and two traps with old lures (either aged 30 days in traps or two years in storage). The differently baited traps were placed on alternate tripods in a row and positions were rotated daily to avoid positional bias. All traps for each experiment were operated over night from 18:00 to 7:00 h. Collection nets were emptied the following morning. The lures were placed in the special lure chambers in the two commercial traps while the lure was placed in the CDC trap within the collection bag 10 cm below the fan. After each trial, the used lures were discarded.

Statistics. The mean numbers of mosquitoes caught per trap (male and female) per night (24 nights) for each trial were calculated from replicates of the experiments and were analyzed using two-way ANOVA followed by a Sidak post-hoc test to rank significance levels. Differences were said to be significant at $P < 0.05$. Analysis was conducted using GraphPad Prism 8.00 for windows (GraphPad Software, La Jolla California, USA). The mean numbers of mosquitoes (±SEM) and P-value of the comparisons are reported in Tables 1 through 3.

RESULTS

The mean numbers of *Cx. quinquefasciatus* females captured by the DynaTrap model DT160 with fresh and 30-day aged ATRAKTA pod lures were 30.0±2.0 and 31.0±2.0, respectively; which were significantly greater than the mean of 18.3±1.7 captured by the control trap with no lure (Fig. 1, Table 1). This represented an increase of 62 and 68%, respectively. The mean numbers of *Cx. quinquefasciatus* females captured by the DynaTrap model DT700 with fresh and 30-day aged ATRAKTA pod lures were 8.7±0.8 and 8.00±0.7, respectively, which were significantly greater than the mean of 3.8±0.5 captured by the control trap with no lure (Fig. 1, Table 2). This represented an increase of 131 and 112%, respectively. The mean numbers of *Cx. quinquefasciatus* females captured

Table 1. The effect of ATRAKTA pod lure type (no lure, fresh lure, or old lure) on mean numbers of *Cx. quinquefasciatus* females and males (± SEM) caught per DynaTrap DT160, DynaTrap DT700, and the CDC trap model 512 during the trials on 14, 10, and 10 consecutive nights from early to mid February-late October 2020.

Trap Model	Sex	Lure comparisons	Mean # ±SEM (Lure 1)	Mean # ±SEM (Lure 2)	Adjusted P Value
DT160	Females	No-lure vs. Fresh Lure	18.3 ± 1.7	30.0 ± 2.0	0.0001
		No-lure vs. Old Lure	18.3 ± 1.7	31.0 ± 2.0	<0.0001
		Fresh Lure vs. Old Lure	30.0 ± 2.0	31.0 ± 2.0	0.9771
	Males	No-lure vs. Fresh Lure	19.7 ± 1.9	20.0 ± 2.0	0.9993
		No-lure vs. Old Lure	19.7 ± 1.9	19.0 ± 2.0	0.9919
		Fresh Lure vs. Old Lure	20.0 ± 2.0	19.0 ± 2.0	0.9771
DT700	Females	No-lure vs. Fresh Lure	3.8 ±0.5	8.7 ±0.8	<0.0001
		No-lure vs. Old Lure	3.8 ± 0.5	8.0 ± 0.7	<0.0001
		Fresh Lure vs. Old Lure	8.7 ± 0.8	8.0 ± 0.7	0.8216
	Males	No-lure vs. Fresh Lure	2.8 ± 0.4	3.0 ± 0.7	0.9945
		No-lure vs. Old Lure	2.8 ±0.4	2.7 ± 0.6	0.9993
		Fresh Lure vs. Old Lure	3.0 ±0.7	2.7 ± 0.6	0.9821
CDC-Candescent	Females	No-lure vs. Fresh Lure	25.0 ±2.8	48.0 ± 5.0	0.0007
		No-lure vs. Old Lure	25.0 ± 2.8	50.0 ± 5.1	0.0002
		Fresh Lure vs. Old Lure	48.0 ± 5.0	50.0 ± 5.1	0.9830
	Males	No-lure vs. Fresh Lure	15.0 ± 2.6	16.0 ± 5.0	0.9978
		No-lure vs. Old Lure	15.0 ± 2.6	16.0 ± 5.0	0.9978
		Fresh Lure vs. Old Lure	16.0 ± 5.0	16.0 ± 5.0	>0.9999

Fresh Lure - direct from the package
Old Lure - 30 days old (in DynaTraps); 2-years old (in CDC Traps)

by the CDC model 512 with fresh and 2-yr aged ATRAKTA pod lures were 48.0±5.0 and 50.0±5.1, respectively, which were significantly greater than the mean of 25.0±2.8 captured by the control trap with no lure (Fig. 1, Table 3). This represented an increase of 94 and 100%, respectively. Ranked trap efficacy in decreasing order for *Cx. quinquefasciatus* females was CDC model 512 > DynaTrap model DT160 > DynaTrap model DT 700.

For each trap type, there was no significant difference between catches of *Cx. quinquefasciatus* females when using fresh or any aged ATRAKTA pod lures (Tables 1-3). Catches of *Cx. quinquefasciatus* males were not significantly affected by using the lures in combination with any trap.

The mean numbers of *Ae. albopictus* females captured by the DynaTrap model DT160 with fresh and 30-day aged ATRAKTA pod lures were 15.8±1.4 and 16.5±1.3, respectively, which were significantly greater than the mean of 7.0±0.7 captured by the control trap with no lure (Fig. 2, Table 2). This represented an increase of 126.9 and 137.6%, respectively. The mean numbers of *Ae. albopictus* females captured by the DynaTrap model DT700 with fresh and 30-day aged ATRAKTA pod lures were 4.8± 0.5 and 4.6±0.4, respectively, which were significantly greater than the mean of 1.6±0.2 captured by the control trap with no lure (Fig. 2, Table 2). This represented an increase of 200.0 and 184.4%, respectively. The mean numbers of *Ae. albopictus* females captured by the CDC model 512 with fresh and 2-yr aged ATRAKTA pod lures were 2.6±0.3 and 2.9±0.4, respectively, which were significantly greater than the mean of 1.4±0.4 captured by the control trap with no lure (Fig. 2, Table 6). This represented an increase of 82.1 and 103.5%, respectively. Ranked trap efficacy in decreasing order for *Ae. albopictus* females was DynaTrap model DT160 > DynaTrap model DT700 > CDC model 512.

For each trap type, there was no significant difference between catches of *Ae. albopictus* females when using fresh or any aged ATRAKTA pod lures (Table 2). Catches of *Ae. albopictus* males were not significantly

Table 2. The effect of ATRAKTA pod lure type on mean numbers of *Ae. albopictus* females and males (± SEM) caught per DynaTrap DT160, DynaTrap DT700, and the CDC trap model 512 during the trials on 14, 10, and 10 consecutive nights from early to mid February-late October 2020.

Trap Model	Sex	Lure comparisons	Mean # ± SEM (Lure 1)	Mean # ± SEM (Lure 2)	Adjusted P Value
DT160	Females	No-lure vs. Fresh Lure	7.0 ± 0.7	15.8 ± 1.4	<0.0001
		No-lure vs. Old Lure	7.0 ± 0.7	16.5 ± 1.3	<0.0001
		Fresh Lure vs. Old Lure	15.8 ± 1.4	16.5 ± 1.3	0.9452
	Males	No-lure vs. Fresh Lure	5.1 ± 0.6	4.9 ± 1.2	0.9995
		No-lure vs. Old Lure	5.1 ± 0.6	5.4 ± 1.1	0.9941
		Fresh Lure vs. Old Lure	4.9 ± 1.2	5.4 ± 1.1	0.9864
DT700	Females	No-lure vs. Fresh Lure	1.6 ± 0.2	4.8 ± 0.5	<0.0001
		No-lure vs. Old Lure	1.6 ± 0.2	4.6 ± 0.4	<0.0001
		Fresh Lure vs. Old Lure	4.8 ± 0.5	4.6 ± 0.4	0.949
	Males	No-lure vs. Fresh Lure	1.1 ± 0.2	1.4 ± 0.4	0.9136
		No-lure vs. Old Lure	1.1 ± 0.4	1.2 ± 0.4	0.9992
		Fresh Lure vs. Old Lure	1.4 ± 0.4	1.2 ± 0.4	0.9728
CDC-Candescent	Females	No-lure vs. Fresh Lure	1.4 ± 0.2	2.6 ± 0.3	0.0099
		No-lure vs. Old Lure	1.4 ± 0.2	2.9 ± 0.4	0.0007
		Fresh Lure vs. Old Lure	2.6 ± 0.3	2.9 ± 0.4	0.8520
	Males	No-lure vs. Fresh Lure	0.5 ± 0.2	0.7 ± 0.2	0.8869
		No-lure vs. Old Lure	0.5 ± 0.2	0.7 ± 0.3	0.9377
		Fresh Lure vs. Old Lure	0.7 ± 0.2	0.7 ± 0.3	0.9991

Fresh Lure - direct from the package
Old Lure - 30 days old (in DynaTraps); 2-years old (in CDC Traps)

affected by using the lures in combination with any trap.

The mean numbers of *An. gambiae* s.l. females captured by the DynaTrap model DT160 with fresh and 30-day aged ATRAKTA pod lures were 31.4±2.4 and 30.0±2.4, respectively, which were not significantly greater than the mean of 29.4±2.3 captured by the control trap with no lure (Fig. 3, Table 3). This represented an increase of 6.5 and 1.9%, respectively. The mean numbers of *An. gambiae* s.l. females captured by the DynaTrap model DT700 with fresh and 30-day aged ATRAKTA pod lures were 4.5±0.5 and 4.3±0.5, respectively, which were not significantly greater than the mean of 4.0±0.4 captured by the control trap with no lure (Fig. 3, Table 3). This represented an increase of 13.0 and 6.3%, respectively. The mean numbers of *An. gambiae* s.l. females captured by the CDC model 512 with fresh and 2-yr aged ATRAKTA pod lures were 6.0±0.7 and 6.0±0.8, respectively, which were not significantly greater than the mean of 4.9±0.5 captured by the control trap with no lure (Fig. 3, Table 3). This represented an increase of 22.5 and 21.4%, respectively. Ranked trap efficacy in decreasing order for *An. gambiae* s.l. females was DynaTrap model DT160 > DynaTrap model DT700 > CDC model 512.

For each trap type, there was no significant difference between catches of *An. gambiae* s.l. females when using fresh or any aged ATRAKTA pod lures (Table 3). Catches of *An. gambiae* s.l. males were not significantly affected by using the lures in combination with any trap.

DISCUSSION

Use of an ATRAKTA pod lure in DynaTraps and CDC traps can significantly increase the numbers of female mosquitoes captured, except for *An. gambiae.*. There was no significant difference between lures that were fresh, and lures aged for 30 days in the traps (or 2 years in the package in the case of CDC traps). The ATRAKTA pod lure significantly increased DynaTrap catches of both nuisance species *Cx. quinquefasciatus* and *Ae. albopictus* females.

Table 3. The effect of ATRAKTA pod lure type on mean numbers of *An. gambiae* females and males (±SEM) caught per Dyna Trap DT160, Dyna Trap DT700, and CDC trap model 512 during the trials on 14, 10, and 10 consecutive nights from early to mid-February-late October, 2020.

Trap Model	Sex	Lure comparisons	Mean # ±SEM (Lure 1)	Mean # ±Sem (Lure 2)	Adjusted P Value
DT160	Females	No-lure vs. Fresh lure	29.4 ± 2.3	31.4 ± 2.4	0.9109
		No-lure vs. Old lure	29.4 ± 2.3	30.0 ± 2.4	0.9973
		Fresh-lure vs. Old lure	31.4 ± 2.4	30.0 ± 2.4	0.9727
	Males	No-lure vs. Fresh lure	11.4 ± 2.2	11.90 ± 2.3	0.9986
		No-lure vs. Old lure	11.4 ± 2.2	11.9 ± 2.4	0.9982
		Fresh-lure vs. Old lure	11.9 ± 2.3	11.9 ± 2.4	>0.9999
DT700	Females	No-lure vs. Fresh lure	4.0 ± 0.4	4.5 ± 0.5	0.8311
		No-lure vs. Old lure	4.0 ± 0.4	4.3 ± 0.5	0.9740
		Fresh-lure vs. Old lure	4.5 ± 0.5	4.3 ± 0.5	0.9791
	Males	No-lure vs. Fresh lure	2.1 ± 0.4	2.0 ± 0.5	0.9982
		No-lure vs. Old lure	2.1 ± 0.4	2.2 ± 0.6	0.9982
		Fresh-lure vs. Old lure	2.0 ± 0.5	2.2 ± 0.6	0.9891
CDC-Candescent	Females	No-lure vs. Fresh lure	4.9 ± 0.5	6.0 ± 0.7	0.5155
		No-lure vs. Old lure	4.9 ± 0.5	6.0 ± 0.8	0.5541
		Fresh-lure vs. Old lure	6.0 ± 0.7	6.0 ± 0.8	>0.9999
	Males	No-lure vs. Fresh lure	1.9 ± 0.5	2.2 ± 0.7	0.9890
		No-lure vs. Old lure	1.9 ± 0.5	2.2 ± 0.7	0.9812
		Fresh-lure vs. Old lure	2.2 ± 0.7	2.2 ± 0.7	>0.9999

Fresh Lure - direct from the package
Old Lure - 30 days old (in DynaTraps); 2-years old (in CDC Traps)

The ATRAKTA pod lure did not make statistically significant increases in catches of *An. gambiae* females. In a 2020 laboratory report by Sierra Research Laboratories, Inc., a similar result was obtained using *An. quadrimaculatus* where the percentage of recovered mosquitoes (between baited and unbaited traps) numbered only 38%. In France in 2011, trap performance when baited solely with octenol was estimated at only 43% in trapping *An. hyrcanus* (Roiz et al., 2012). Essen and colleagues (1994) reported differential attraction of *Aedes* and *Culex* mosquitoes to light and octenol baited CO^2 traps.

Carbon dioxide is sometimes used as a general attractant (Newhouse et al. 1966) and there are a number of chemical lures on the market that will enhance the attraction of mosquito traps (Bernier et al. 2008). Some of these lures will attract certain mosquito species more than others (Essen et al. 1994; Burkett et al. 2001). The ATRAKTA pod lure was selective in its attraction of *Cx. pipiens quinquefasciatus* and *Ae. albopictus*.

Many mosquito traps, including the ones in the study, use some type of light as an attractant (Kline 1994; Ponlawat et al. 2017). A recent study showed that a new model of DynaTrap, DT 2000, baited with ATRAKTA collected significantly higher numbers of adult mosquitoes and non-targets, compared with the CDC light trap baited with the same lure (Acevedo et al. 2020). It is notable that the DynaTrap DT160 caught significantly more females than the DT700 regardless of lure type. The DynaTrap DT160, utilizes a 3.5W±10% circular Cold Cathode Fluorescent Light (CCFL) source that produces a wavelength of 365 nm±5 nm, drawing 0.4A/hour from a 12V battery, whereas the DT700 uses UV emanating from 3 small LED bulbs. The better performance of the DynaTrap DT160 could be affected by the differences in the type of UV source, as well as the configuration of the bulb.

In conclusion, ATRAKTA pod 3-part lures can be used to boost trap catches of common nuisance mosquito females, in some cases by well over 100% either fresh

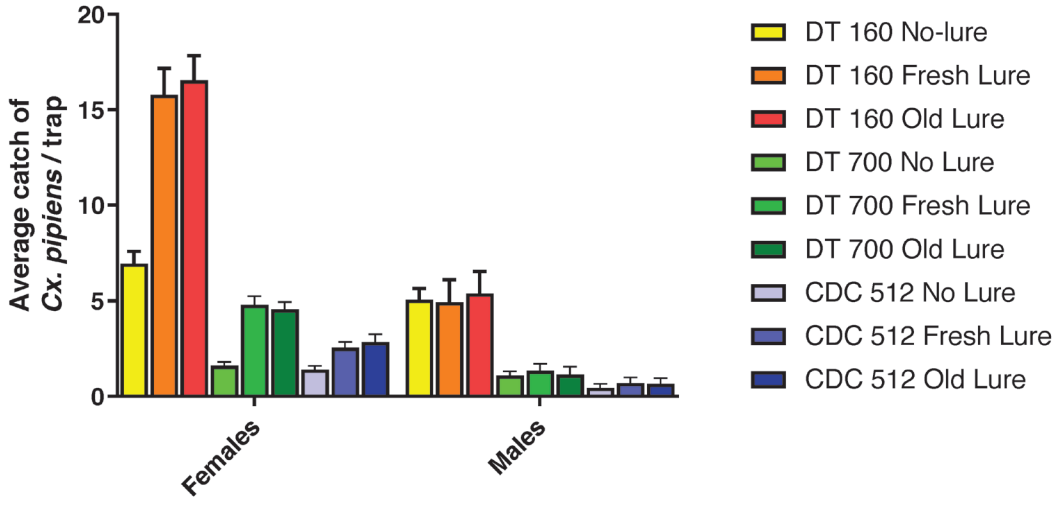

Fig. 2. Effect of the ATRAKTA pod lure on DynaTrap DT160, DT700 and CDC model 512 trap catches of *Ae. albopictus*.

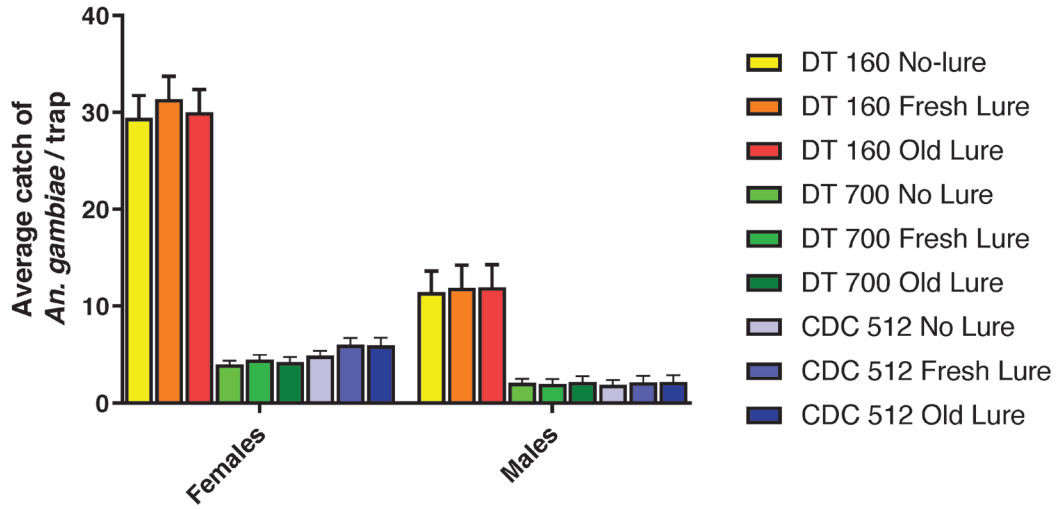

Fig. 3. Effect of the ATRAKTA pod lure on DynaTrap DT160, DT700 and CDC model 512 trap catches of *An. gambiae* s.

out of the bag, under field conditions, or after prolonged storage periods.

ACKNOWLEDGEMENTS

The authors wish to thank participating communities in Bamako and Kenieroba, Mali. The trials were in part supported by the ICEMR Vector supplement Grant Number 5U19AI089672.

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FIELD EVALUATION OF TALSTAR (BIFENTHRIN) RESIDENTIAL BARRIER TREATMENTS ALONE AND IN CONJUNCTION WITH MOSQUITO MAGNET LIBERTY PLUS TRAPS IN CEDAR KEY, FLORIDA

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ABSTRACT

The effectiveness of bifenthrin applications to vegetation with and without commercial mosquito traps (Mosquito Magnet Liberty Plus) was evaluated against *Culicoides* biting midges in a residential coastal area located in Cedar Key, Florida. Efficacy evaluations were determined by surveillance trap collections and modified landing rate counts. In general, all treatments provided significant reduction from *Culicoides* biting midge pressure when compared with untreated yards with no traps (control). However, the combination of bifenthrin and Liberty Plus traps proved to be the most successful in reducing *Culicoides* compared with yards with only a Liberty Plus trap. Yards treated with bifenthrin alone or in combination with the Liberty Plus trap were more successful than controls, suggesting that *Culicoides* biting midge population suppression may be obtained through barrier application alone.

Key Words: *Culicoides*, biting midges, bifenthrin, barrier, traps

INTRODUCTION

Insecticides when applied as barrier sprays to vegetation has proven effective in the suppression of mosquitoes (Fulcher et al. 2015) and can be an effective part of an Integrated Mosquito Management plan (Richards et al. 2017). However, such applications have limited success against *Culicoides* biting midges. Kettle (1949) attempted to control *Culicoides impunctatus* (Goetghebuer) in Scotland by providing a barrier spray of DDT to vegetation at two pounds per acre. Unfortunately, this effort provided no control. Previous studies have reported success with fogging applications of organophosphates or DDT (Trapido 1947, Bruce and Blakeslee 1948). Conversely, Linley and Davies (1971) concluded that fogging was a waste of effort best reserved for emergencies when a *Culicoides* population reaches intolerable levels.

Biting midge suppression has been shown when adulticiding aerially using ultra low volume (ULV) applications (Breidenbaugh and Szalay 2010). However, these applications are limited to equipment availability, operating costs, and duration of effectiveness. Madden et al. (1946) were able to control *C. furens* (Poey) for three days using 0.28 kg DDT per hectare when applied aerially. Giglioli et al. (1980) reported 95% reduction when applying ULV fenitrothion aerially. Haile et al. (1984) reported that aerial ULV applications with naled provided 99% control for up to three days; the authors concluded that *Culicoides* control could be obtained with sufficient dose and frequency using this product. Linley and Jordan (1992) were able to provide 90% control for adult

Culicoides populations when applying insecticide using an aerial ULV machine.

The pesticide industry has moved to more environmentally safe pesticides such as pyrethroids to accommodate Environmental Protection Agency (EPA) regulations. Furthermore, tests have shown that *Culicoides* have a higher mortality rate when exposed to pyrethroids than organophosphates (Kline et al. 1981, Floore 1985). This suggests that the use of pyrethroids could be a vital part of today's IPM program for *Culicoides*. Standfast et al. (2003) used bifenthrin to treat various home external resting surfaces in River Heads, Hervey Bay, Queensland, Australia. Applied as a coarse spray, bifenthrin resulted in a 75 to 97% reduction in biting midge numbers in the first month and a 65% reduction at 6 weeks post-treatment. The authors concluded that the bifenthrin applications were successful in controlling biting midge numbers during peak emergence while satisfying federal concerns about broad-scale insecticide application to sensitive areas.

Adult mosquito traps are commonly used for mosquito surveillance to evaluate treatment methods or monitor mosquito population abundance (WHO 2013, Li et al. 2016, Bazin and Williams 2018, Wilke et al. 2019)). Mosquito traps have also been used with some success as a control technique to reduce mosquito populations using a propane fueled Mosquito Magnet (MM) Pro (Kline 2006). Moreover, mosquito trap collections have recorded large numbers of *Culicoides* biting midges in the capture nets of MM Freedom and Liberty Plus traps (Lloyd et al. 2008) posing the question; can mosquito traps, alone, be used to reduce *Culicoides* biting midge populations?

The objective of this study was to evaluate the efficacy of treating shrubbery surrounding homes with Talstar (7.9% bifenthrin) only or MM Liberty Plus traps alone as well as their combination against *Culicoides* populations

Study Site. The study site consisted of a residential neighborhood (Rye Key; 29.153982, -83.0460009) in Cedar Key, Florida. Rye Key is a 5.91 ha island surrounded by the Gulf of Mexico with extensive inlets with black needle rush (*Juncus* spp.) and cordgrass (*Spartina* spp.).marsh located at the northeastern tip of Cedar Key. This site was chosen because of its previous history of consistently producing large populations of *Culicoides* (Lloyd et al. 2008). In addition, access to the neighborhood was limited by an electronic gate reducing the chance of vandalism or theft of equipment. The surrounding flora associated with each site was similar.

Treatments. Treatment one was a MM-Liberty Plus® trap (Liberty Plus), baited with an octenol cartridge manufactured by Wood Stream Corporation (Lititz, PA). The Liberty Plus trap was set up per manufacturers recommendations to run continuously during the study. The Liberty Plus is a propane powered, counterflow geometry trap that encapsulates a hybrid power fueled by propane that generates heat, moisture, and approximately 550 ml/min combusted CO_2. The average surface temperature of this trap was 37.1°C with plume temperatures between 33.3 to 40.6 °C. The Liberty Plus has a push-button start with lights that indicate when the machine is operating and if service is needed.

Treatment two was a Talstar® (7.9% bifenthrin) (FMC, Philadelphia, PA) application to shrubbery surrounding the home. The insecticide was applied at max label rate (29.6 milliliters per 3.8 liters, 3.8 liters per 92.9 square meters) using a Solo® (Newport News, VA) backpack sprayer delivering the insecticide dilution in low volume. Retreatment of applications following manufacturer's label instructions of once every four weeks. Treatment three was a combination of the Liberty Plus trap as described in treatment one and Talstar application as described in treatment two. Treatment four was a control with locations under normal

conditions without traps or insecticide. The study was conducted between March and October 2009. All residences in the study were at least 50 m from one another.

Data Collection. Two different assessments were incorporated during the study at each residential location. One surveillance sticky trap was placed at each location between the house and treatment. Traps were constructed of a 15.2 x 15.2 cm sheet of Web Trap® adhesive papers (Applica, Miami Lakes, FL) mounted onto a wooden stake secured 1m above the ground and baited with 0.45 kg of dry ice housed in a cooler on the ground (John W. Hock, Gainesville, FL). Collections were obtained every 1-2 days to record biting midge capture for that time period. The surveillance sticky trap was baited with dry ice every 1-2 days and placed at the location before dusk. A flyswatter count was taken every 1-2 days per week during the study at each residential location for 3 minutes using a 15.2 x 15.2 cm section cut from a sheet of Web Trap® adhesive paper (Applica, Miami Lakes, FL) and used to swat pursuing *Culicoides* from the air.

The Liberty Plus trap nets were collected and replaced with a replacement net 1-2 days per week throughout the study. Each Liberty Plus net removed from traps were placed into individual one-gallon plastic Ziploc bag, and any insects inside traps but outside nets were vacuumed and placed inside the respective Ziploc bag. Each Ziploc bag was labeled individually with location, date, and trap identifier. The propane tanks were changed every 18 days. The octenol cartridges were changed every 21 days.

Swatting count were conducted every 1-2 days per week from each location, covered in clear cellophane wrap and labeled with location, date, and treatment information. Surveillance sticky papers were collected every 1-2 days per week from each location and replaced with a new paper. The collected surveillance sticky papers were covered in clear cellophane wrap and labeled with location, date, and treatment information.

Once collections were returned to the laboratory they were stored in a -20 °C freezer until processed. If the number of *Culicoides* captured was estimated to be more than 500, an aliquot was extrapolated from the total capture and weighed. The weight of the aliquot was divided into the total captured weight and the quotient was multiplied by the number of *Culicoides* identified and counted in the aliquot. If the number of *Culicoides* was estimated to be below 500, the entire collection was identified and counted. Samples were identified to species and counted (Blanton and Wirth 1979).

Data Analysis. Data were initially normalized by conversion to $\log_{10}$ (n+1) then subjected to ANOVA (SAS 2003) using the following model statements: Method = Swatting Treatment Week; Method = Sticky Treatment Week; Treatment = Control Method Week; Treatment = Liberty Plus Method Week; Treatment = Liberty Plus/ Talstar Method Week; Treatment = Talstar Method Week; where dependant variables represented numbers of biting midges captured. Method was one of the surveillance methods used to determine biting midge pressure, treatment was one of the four assigned control measures, and week was one of the 20 trapping weeks of the study. Means were separated with the Ryan-Einot-Gabriel-Welsch Multiple Range Test (REGWQ), and unless otherwise stated, $P<0.05$ (SAS 2003). Although $\log_{10}$ (n+1) values were used for the analyses, actual means are reported in the text, and tables.

RESULTS

Analysis of data by calendar week yielded no significant difference among treatments and weeks for all *Culicoides, C. furens, and C. mississippiensis* captured. Significant differences in the sticky surveillance and swatting count methods used to survey all *Culicoides* from March to October 2009 were observed ($F= 18.10$, $df= 3, 19$, $p< 0.0004$). Sticky sur-

veillance trap method consistently captured more *Culicoides* than the swatting counts.

Four species of *Culicoides: C. barbosai* (Wirth and Blanton), *C. furens* Poey, *C. melleus* (Coquillett), and *C. mississippiensis* Hoffman were collected from sticky traps, flyswatters, and MM Liberty Plus traps (Tables 4-1, 2). There was no significant difference among treatments and weeks or surveillance methods and weeks when overall total abundance was considered on sticky traps. However, all treatments, significantly reduced total abundance of midges compared with controls from those traps (Table 4-3). However, total *Culicoides* reduction was not significantly different between Talstar application with and without the Liberty Plus. Two major species (*C. furens* and *C. mississippiensis*) were collected in large enough numbers to be statistically analyzed. *Culicoides furens* reduction was similar to that for previously mentioned for total *Culicoides* species on sticky traps. All treatments significantly reduced *C. mississippiensis* abundance compared with controls but were not different from one another (Table 4-3). Also, there was no difference in the number of *Culicoides* collected in Liberty Plus traps with or without the Talstar application (Table 4-4).

Significant differences in *C. furens* captured on sticky traps among treatments were observed ($F= 11.95$, $df= 3, 79$ $p< 0.0001$). The sticky surveillance trap located at the Talstar treatment area caught less *C. furens* than the control and Liberty Plus treatment areas. There were no differences between surveillance sticky traps located at the Talstar and Liberty Plus + Talstar areas. Significant differences in total *C. mississippiensis* captured among treatments (Control, Liberty Plus, Liberty Plus + Talstar, Talstar) from March to October 2009 were observed ($F= 4.45$, $df= 3, 79$ $p< 0.0071$). The surveillance sticky trap located at the control treatment area captured more *C. mississippiensis than* any surveillance sticky trap located at the other treatment areas. There were no differences in surveillance sticky traps located at the Talstar, Liberty Plus + Talstar, and Liberty Plus treatment areas.

DISCUSSION

The objective of this study was to determine if an insecticide barrier treatment alone or in conjunction with commercially available traps could provide protection against host-seeking biting midges. Providing a residual insecticide barrier around an area for protection against mosquitoes is not a novel technique (Ludvik 1950, Quarterman et al. 1955, Helson and Surgeoner 1983, Anderson et al. 1991, Perich et al. 1993, Frances 2007, Trout et al. 2007, Cilek 2008, Britch et al. 2009, Qualls et al. 2012, Bibbs et al. 2016). However, barrier treatments with a residual insecticide for protection against *Culicoides* has been understudied (Kettle 1949, Standfast 2003) and the author is unaware of any other study that evaluates the combination of commercial traps and barrier treatments to protect against *Culicoides*.

In general, all treatments provided significant reductions from biting midge pressure when compared to the control. However, the two treatments utilizing Talstar were more successful (89-98% reduction) than the Liberty Plus trap treatment alone (68% reduction). Previous studies have shown similar success using Talstar against mosquitoes to protect military tents (Frances 2007), park recreation areas (Cilek 2008) and even desert environments with sparse vegetation (Britch et al. 2009). In this study, the insecticide barrier treatment provided the greatest reduction in *Culicoides* numbers captured on the surveillance traps. The combination treatment provided the next best reduction. The Liberty Plus trap provided the least *Culicoides* reduction, but was still significantly better than the control treatment. Standfast (2003) reported similar success in Australia (97% reduction) when they treated all surfaces on and surrounding the homes of their treatment sites with Bistar (bifenthrin). The authors conducted one bifenthrin application on vegetation, fence panels, and walls of the homes that they intended to protect from *Culicoides*. The authors monitored the population reporting a decrease in effectiveness (60% reduction) 4-6 weeks after treatment. We were able to produce our results with treatment of the surrounding vegetation alone indicating

that it is possible to receive near-maximum biting midge suppression with reduced insecticide exposure for up to 4 weeks.

When total *Culicoides* and individual species were evaluated, there were significant differences in surveillance methods used, and treatment effects. Swatting counts were not effective at assessing the host-seeking biting midge population. The short duration (3 minutes) assigned to the swatting counts was not sufficient for determining the true host seeking biting midge pressure. In addition, it is not reasonable to spend the time that seems to be required for pressure assessment. Furthermore, timing and climatic events can severely skew pressure assessments that are conducted for short periods of time. The modified sticky surveillance trap was more efficient with pressure assessment and less labor intensive. This method provided a survey for the entire time that a treatment was implemented. However, there is a substantial cost involved ($1.50 per kilogram) with refilling dry-ice every 24-48 hours. The sticky surveillance trap is an efficient tool for biting midge pressure assessment, but needs to be refined to reduce costs.

The Liberty Plus trap used in the combination treatment captured more *Culicoides* than the Liberty Plus trap alone. It is uncertain as to why this phenomenon occurred. Although the trap capture results are not significant, it is important to discuss the possibilities resulting in increased trap capture for the combination treatment. It is possible that the insecticide treatment dramatically reduced the amount of resting harborage available due to behavioral avoidance; therefore the *Culicoides* will be more likely to fly towards a trap that produces an attractant plume rather than rest on a surface treated with insecticide, creating a push-pull protection system. Another possibility is the addition of the Liberty Plus trap in combination with the insecticide treatment is attracting or pulling in the biting midges from outside the protected area actually attracting biting midges that may not have normally traveled to the home.

The results from this study suggest that the Liberty Plus trap, Talstar and Talstar/Liberty Plus (combination) treatment will reduce the *Culicoides* population pressure around homes. However, to maximize suppression and response time for protection from *Culicoides*, insecticide treatment alone is the most efficient and economically effective population management technique. Commercial traps may have potential for long term (3-5 years) control programs by providing an alternative control solution to manage insecticide resistance and potentially decreasing a pestiferous population over time. Further evaluations utilizing insecticide applications and commercial traps as a combination treatment are needed.

Table 4-1. Total number of *Culicoides* species caught in Cedar Key, FL. from March to October 2009 using four modified sticky surveillance traps and swatting counts.

Species	Sticky surveillance traps	Swatting paper	% of total *Culicoides* captured
C. furens	56,779	1,709	88.85
C. mississippiensis	6,328	251	9.90
C. barbosai	532	0	0.84
C. melleus	262	0	0.41
Grand Total	63,901	1,960	100

Table 4-2. Total number of *Culicoides* species caught in two MM-Liberty Plus traps in Cedar Key, FL from March through October 2009. n = 60

Species	No. of *Culicoides spp.* captured	% of total *Culicoides* Captured
C. furens	77,910	92.63
C. mississippiensis	4,898	5.82
C. barbosai	843	1.02
C. melleus	449	0.53
Grand Total	84,100	100

Table 4-3. Mean number of *Culicoides* species captured on modified sticky surveillance traps placed at each treatment site in Cedar Key, FL from March through October 2009. n = 80; p< 0.05

Treatments	Total *Culicoides*	*Culicoides furens*	*Culicoides mississippiensis*
Control	2024 ± 440a	1905 ± 411a	890 ± 325a
Liberty Plus	864 ± 322b	234 ± 79b	205 ± 79b
Liberty Plus + Tastar	45 ± 13bc	38 ± 11bc	119 ± 46b
Talstar	25 ± 11c	29 ± 20c	6 ± 3b

Within a column, means followed by the same letter designate no significant difference 223 in collections among treatments (alpha=0.05).

n= the number of trap replicates for the corresponding time period.

Within a column, degree of freedom for species captured error for each treatment is 79; treatment df = 3

Data were log (n+1) transformed prior to analysis using an ANOVA with trapping method (sticky surveillance trap)and treatment in the model. Significance among treatments analyzed with Ryan-Einot-Gabriel-Welsch multiple range test.

Table 4-4. Mean trap capture comparison between the MM-Liberty Plus and MM-Liberty Plus+ Talstar treatments in Cedar Key, FL from March through October 2009. *n* = 60; p< 0.05

Species	MM-Liberty Plus	MM-Liberty Plus+ Talstar
Culicoides spp.	1,972 ± 520	2,232 ± 462
C. furens	1,948 ± 516	2,184 ± 460
C. mississippiensis	24 ± 14	48 ± 23

Within a row, there were no significant differences between MM-Liberty Plus trap collection means (alpha=0.05).

n= the number of trap replicates for the corresponding time period.

Within a column, degree of freedom for species captured error is 59; trap df = 1.

Data were log (n+1) transformed prior to analysis using an ANOVA with trapping period (method) and trap treatment in the model. Significance between traps analyzed with Ryan-Einot-Gabriel-Welsch multiple range test.

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SEMI-FIELD EVALUATION OF ULTRA-LOW VOLUME (ULV) GROUND SPRAY OF AQUALUER® 20-20 AGAINST IRRADIATED *AEDES AEGYPTI*

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Subject Editor: Keira J. Lucas

ABSTRACT

Sterile insect technique (SIT) using irradiated mosquitoes is an effective control method capable of being assimilated into integrated vector management (IVM) programs. Chemical control of mosquitoes using ultra-low volume (ULV) spray applications of pyrethroid and organophosphate insecticides is already an essential component of IVM programs. Prior to their release in nature, irradiation of mosquitoes for SIT use can significantly impact the mosquito's biology, specifically its host-seeking and feeding behavior. Little is known about how radiation exposure might impact a mosquito's susceptibility to pyrethroid insecticides. The present study was carried out to evaluate the influence of Aqualuer® 20-20 ULV applications on irradiated *Aedes aegypti*. Caged mosquito trials indicated that both male and female irradiated *Ae. aegypti* were as susceptible as their non-irradiated counterparts of the same population to Aqualuer 20-20 ULV application, with the highest mean percent mortalities achieved at the first 24h post-treatment period at both 30.5 m and 61 m downwind of the spray application path.

Key Words: Sterile insect technique, irradiated mosquitoes, *Aedes aegypti*, ULV, integrated vector management

INTRODUCTION

Aedes aegypti (L.) is the principal vector of several emerging and re-emerging arboviral disease agents including dengue, chikungunya, Zika and yellow fever viruses in tropical and subtropical regions worldwide (Bonica et al. 2019, Gubler 2002, Higgs and Vanlandingham 2015, Kraemer et al. 2015, Reiskind et al. 2008, Thavara et al. 2009, Weaver and Reisen 2010). In the absence of effective vaccines or drugs to prevent or treat these diseases, the most effective strategy has been to disrupt the virus transmission cycle by reducing the frequency of human-vector contact (Wilder-Smith et al. 2017). Contemporary vector control methods, such as thermal or ULV space spray and larvicide applications to reduce adult and larval vector populations and physical methods used to reduce breeding sites or to deter vector contact with humans, have limited ability to effectively control vector populations. These methods are best applied as part of an integrated vector management (IVM) program (Esu et al. 2010, Lima et al. 2015, Marini et al. 2019), in which the chemical and non-chemical vector control methods are appropriately integrated to achieve the optimal effectiveness (WHO 2020). In fact, there is evidence that currently used insecticide applications have led to the development of insecticide resistance in *Ae. aegypti* (Deming et al. 2016, Ishak et al. 2015). Therefore, the need for novel complementary vector control tools that are effective, sustainable, and environmentally benign is becoming a high priority (Fernández-Salas et al. 2015).

Sterile insect technique (SIT) is an environmentally safe control method, being species specific and without leaving any chemical residues (Alphey et al. 2010). One component of SIT involves chemo-sterilization of male insects. It requires colonization

and mass rearing of the target insect, the sterilization of large numbers of the reared male insects by ionizing irradiation using gamma- or X-rays and their subsequent periodic release into the target area, where they compete with wild males for mating with wild females. Those wild females lay only sterile eggs which in turn leads to suppression of the population. Irradiation-based SIT has been used successfully since the 1930's to control many agricultural and other pests such as Mediterranean fruit fly (*Ceratitis capitata,* Weidemann), screw worm (*Cochliomyia hominivorax,* Coquerel), pink bollworm (*Pectinophora gossypiella*, Saunders), and tsetse fly (*Glossina austeni*, Wiedemann) (Cayol et al. 2002, Dowell et al. 1998, Henneberry 1994, Vargas-Teran et al. 2005, Vreysen et al. 2000). Studies have demonstrated that this technique has been successfully used against several mosquito species including *Ae. albopictus* (Skuse), *Anopheles albimanus* (Weidemann) and *Culex quinquefasciatus* (Say) (Bellini et al. 2013, Benedict and Robinson, 2003, Lofgren et al. 1974, Patterson et al. 1970). The optimal use of SIT in vector control should be within an IVM program, with the potential to reduce the vector population below an arbovirus transmission threshold (Alphey et al. 2010).

Ultra-Low Volume (ULV) ground-spray application of adulticides is often a key and effective component of IVM programs to reduce arbovirus vector and nuisance biting mosquitoes (Faraji 2016). Pyrethroids, such as permethrin, are commonly used in ULV adulticide programs (EPA 2019) due to their relative stability and low toxicity to a wide range of insects at low application rates used for mosquito control applications (Elliott 1976). ULV spray of Aqualuer® 20-20 (20.6% permethrin and 20.6% piperonyl butoxide; AllPro Inc., St. Joseph, MO) is one of the main components of the IVM program of the Anastasia Mosquito Control District (AMCD), located in St. Augustine, Florida.

In 2017-2018, AMCD conducted regular ULV applications of Aqualuer 20-20 in response to service requests stating that residents were concerned about an abundance of the nuisance salt marsh mosquito, *Ae. taeniorhynchus* (Wiedemann). The service requests coincided with areas where irradiated male *Ae. aegypti* were being released for SIT trials. However, with SIT trials in progress, very little research had been done to investigate any potential discrepancy in the effects of ULV sprays on released irradiated male mosquitoes compared to wild males of the same species and the implication on how this could impact future SIT releases. The present study was carried out to determine the impact of Aqualuer 20-20 ULV ground application on irradiated *Ae. aegypti*. It would help to determine if ULV spraying could be used to selectively reduce wild males within a SIT program to increase the chances of remaining wild females mating with irradiated males thus warranting the incorporation of SIT into the IVM program.

MATERIALS AND METHODS

Semi-field trials (WHO 2009) were conducted with laboratory-reared, irradiated and non-irradiated, male and female *Ae. aegypti* of the same population (St. Augustine strain) in a 90 m x 90 m grid test site at AMCD. Mosquitoes were reared in insectaries at the United States Department of Agriculture's Center for Medical, Agricultural & Veterinary Entomology (USDA-CMAVE), in Gainesville, Florida. The incubators (Percival Scientific, Perry, IA) were maintained at 28° ± 1°C, 70% relative humidity (RH) and 14:10 L:D photoperiod. Immatures were fed on a diet of pulverized tetramin *ad libitum* and adults were fed *ad libitum* with 10% sucrose solution soaked in cotton balls. Male and female *Ae. aegypti* pupae were irradiated with 50 Gray (Gy) by γ-radiation using a Gammator M (Radiation Machinery Corp., Parsippany, NJ) containing a cesium-137 source that generated 8.8 Gy/min. The radiation doses applied to pupae were 0 and 50 Gy, with the 0 Gy acting as a control. Radiation doses were checked with alanine films applied to petri dishes with pupae for every dose.

Nine sentinel cage poles were distributed in the treatment plot in a 3 x 3 grid with 30.5 m separations between each row. The sentinel poles were placed at 30.5 m, 61.0 m and 90.4 m downwind of the spray-truck path (Fig. 1). Additionally, three control sentinel cage poles were positioned upwind of the spray zone. A weather station (WatchDog 2550, Spectrum Technologies Inc., Aurora, IL) was placed in the treatment plot to monitor wind speed and wind direction to select time of application and the direction of the spray-truck path. Temperature and RH were recorded immediately before and after each application. Twenty mosquitoes from all 4 groups (irradiated males and females, and non-irradiated males and females) were aspirated into 4 separate cylindrical screened paper cages (10 x 4 cm) to make a set. Each set of 4 cages were mounted on the sentinel cage poles approximately 1.2 m above ground level in the treatment plot. A rotating impinger (Leading Edge Associates Inc., Fletcher, NC) with two Teflon-coated glass slides was fixed to each sentinel cage pole for the verification of insecticide reach. A truck-mounted single-nozzle ULV cold aerosol sprayer (Guardian 95ES, Adapco, LLC, Sanford, FL) was driven at 16 km/h perpendicular to the wind direction with an application rate of 2.9 to 3.5 L/hectare and droplet size (mass median diameter) of 25.7 microns. Dilution of the insecticide was 1 part Aqualuer 20-20 to 9 parts water. The spray-tuck started 30.5 m prior to the first cage pole of the row and was stopped 30.5 m after the last cage pole to ensure the spray coverage was sufficient. Paper cages and Teflon slides were collected and brought back to the laboratory 15 min post application. The three sentinel control poles with cages were placed upwind of the spray zone for 15 min just prior to starting the treatment, collected, and returned to the laboratory. All the cages were provided with a cotton pad soaked in 10% sucrose solution and the number of knocked down mosquitoes in each cage was recorded after 1 h. Mortality counts were taken at 24 h and 48 h post application. Three successful replications were conducted in June/July 2019 between 0730 to 0930 with at least one week separating the evaluations.

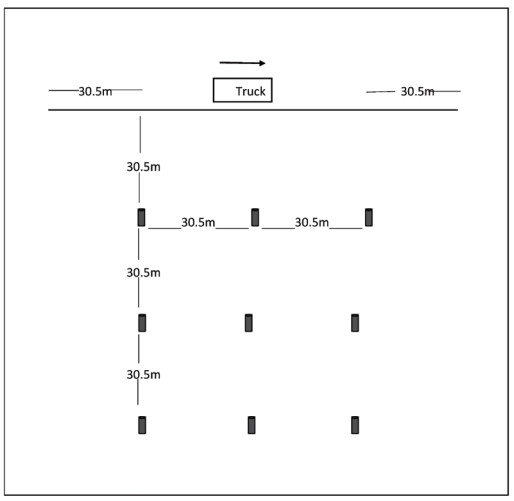

Figure 1. Layout of sentinel cage poles and the direction of spray-truck path.

Data were analyzed using SPSS (IBM® SPSS® statistics, V. 20). A Kruskal-Wallis test and Mann-Whitney U test was used appropriately for comparisons because the Shapiro-Wilk normality test could not confirm the normal distribution of data sets.

RESULTS

Immediate effects of Aqualuer 20-20 ULV application on irradiated and non-irradiated adult mosquitoes were determined by comparing percent knockdown between treatment and control groups at 1 h post application. First, the percent knockdown of the four groups - irradiated and non-irradiated, control and treatment - were analyzed separate to determine any significant differences between the sexes. Since there were no statistically significant differences between the sexes in any of the groups ($P>0.05$ for all), data for sexes were pooled to compare the effect of the distances from the spray path. Percent knockdown showed a highly significant difference among the downwind distances of both irradiated ($\chi^2_{(2)} = 18.98$, $P<0.001$) and non-irradiated mosquitoes ($\chi^2_{(2)} = 14.55$, $P<0.01$). Significantly higher knockdown was observed at 30.5 m downwind than at 61 m (Mann-Whitney $U = 81.5$, $P<0.05$ for the irradiated mosquitoes and $U = 91.0$, $P<0.05$ for

the non-irradiated mosquitoes). Knockdown was higher at 61.0 m than 90.4 m only in irradiated mosquitoes (U = 92.0, P<0.05). As there were significant differences in knockdown between downwind distances, control and treatment groups were compared at different distances to determine the immediate effect of the ULV application. The immediate effects of Aqualuer 20-20 ULV application on both irradiated and non-irradiated mosquitoes were statistically significant only at 30.5 m downwind of the spray path (U = 55.0, P<0.01 and U = 52.5, P<0.01, respectively (Fig. 2).

Forty-eight-hour post application mean percent mortalities of the treatment group were below 25% while those in the control group were below 4%. Although there were no statistically significant differences in mortality between the two sexes, corresponding mortality of males was always higher than that of treated females while it was lower than the control females (Table 1). Once the mortality data of the two sexes was pooled, the differences in percent mortality of the treatment group were significant among the downwind distances [$\chi^2_{(2)}$ = 9.15, P<0.05 and $\chi^2_{(2)}$ = 7.72, P<0.05 for irradiated and non-irradiated mosquitoes, respectively]. The observed differences were only between the 30.5 m and 90.4 m downwind distances (U = 75.5, P<0.05 and U = 67.5, P<0.05 for irradiated and non-irradiated mosquitoes, respectively). Delayed effects of Aqualuer 20-20 ULV application on irradiated and non-irradiated mosquitoes, ascertained by comparing mortality at 48 h post application between the treatment and control groups were statistically significant at 30.5 m (U = 56.0, P<0.001 and U = 89.0, P<0.001 respectively) and 61.0 m (U = 50.5, P<0.05 and U = 84, P<0.01 respectively) downwind of the spray path. There were no statistically significant differences in mortality between the treatment groups of the irradiated and non-irradiated mosquitoes at any of the distances (Fig. 3). Percent mortalities were significantly higher at the first 24 h period than at the second 24 h period at both 30.5 m (U = 83.5, P<0.05 for irradiated mosquitoes; U = 41.0, P<0.001 for non-irradiated mosquitoes) and 61.0 m (U = 100.0, P<0.05 for irradiated mosquitoes; U = 81.5, P<0.01 for non-irradiated mosquitoes) downwind of the spray path.

Environmental temperature, RH and wind speed at both control and treatment sites for all replicates ranged between 25.5-29.4°C, 69-88% and 3.2-8.0 km/h, respectively. Teflon-coated slide readings recorded that the droplet density ranged from <10 droplets/mm² (on slides placed at 30.5 m) to <5 droplets/mm² (on slides placed at 91.4 m).

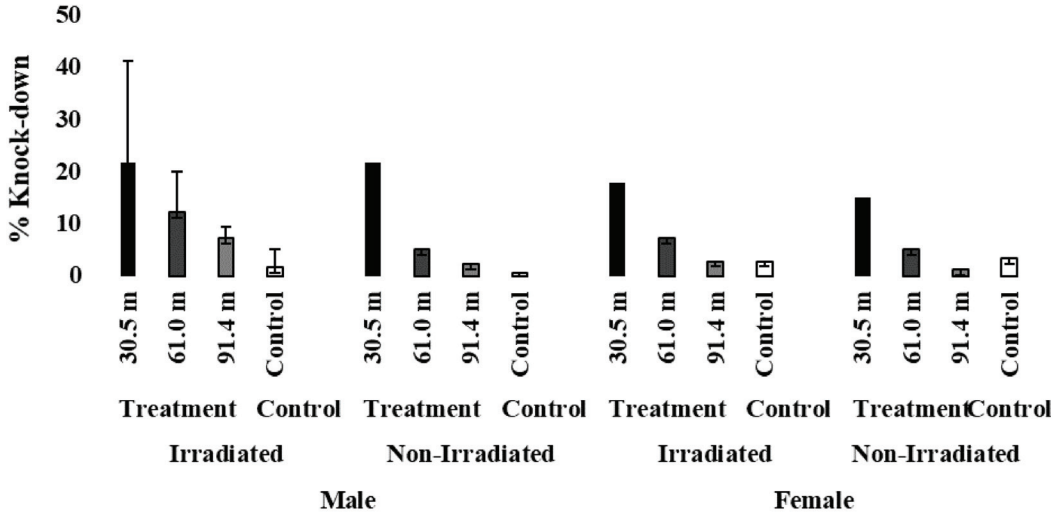

Figure 2. One-hour post-treatment knockdown between treatment and control groups of irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different downwind distances.

Table 1. Forty-eight-hour post-treatment mortalities of male and female irradiated and non-irradiated *Ae. aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different distances (mean ± standard error).

	Irradiated *Ae. aegypti*		Non-irradiated *Ae. aegypti*	
	male	female	male	female
30.5 m	21.70 ± 5.59	17.78 ± 5.40	26.67 ± 7.5	17.22 ± 7.08
61.0 m	12.22 ± 5.84	7.22 ± 1.88	11.11 ± 3.41	10.00 ± 4.17
90.4 m	7.22 ± 2.06	2.78 ± 0.88	6.11 ± 2.00	3.89 ± 2.17
Control	1.60 ± 0.83	2.78 ± 0.88	0.56 ± 0.56	3.33 ± 1.17

DISCUSSION

This study demonstrated that both irradiated and non-irradiated *Ae. aegypti* mosquitoes were equally susceptible to Aqualuer 20-20 ULV applications at least up to 61.0 m downwind. The insecticide application did not show any difference in mortality between sex, and the highest mortality was achieved within 24 h post-treatment. This indicates that Aqualuer 20-20 ULV applications would immediately knockdown both male and female *Ae. aegypti* mosquitoes in the environment without regard to sterilization status. Since it is imperative that released SIT male mosquitoes should have a maximum lifespan (Culbert et al. 2020) to disperse well in the environment, find wild females cohorts, and mate successfully, the simultaneous use of ULV applications to control other species in the same area as the release site would likely negatively impact the efficacy of the SIT release. Because of this, SIT might be better at targeting the last remaining vectors rather than targeting when populations are elevated. As the effectiveness of the SIT program is related to the ratio of released males to wild fertile females, and released sterile males will actively seek out wild females, SIT can target these remaining individuals and reduce the population further, probably from low to zero (Alphey et al. 2010).

Low mortality rates (<25%) observed during the Aqualuer 20-20 ULV spray could be due to several reasons: spray trials might

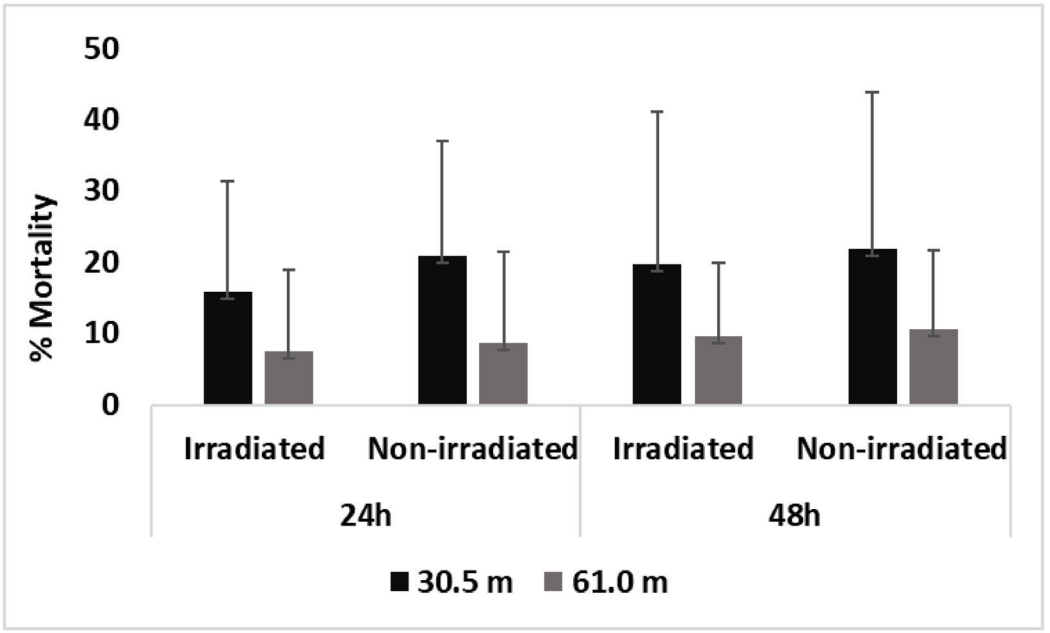

Figure 3. Cumulative mortality between irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different downwind distances.

Table 2. Comparison of cumulative mortality between treatment and control sets of irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray (Mann-Whitney test).

	Irradiated *Ae. aegypti*						Non-irradiated *Ae. aegypti*					
	30.5 m		61.0 m		90.4 m		30.5 m		61.0 m		90.4 m	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
U	60.0	56.0	95.5	89.0	145.5	115.0	48.0	50.5	87.0	84.0	144.0	117.5
p	0.001	0.000	0.019	0.013	0.519	0.104	0.000	0.000	0.010	0.008	0.502	0.116

U = Mann-Whitney U, p = significance level

have been affected by the sub-optimal wind speeds or the tested strain of *Ae. aegypti* might have developed resistance to pyrethroids. According to WHO (2009), outdoor small-scale insecticide spray applications should not take place when wind speeds falls below 3 km/h. Pesticide drift potential is lowest at wind speeds between 4.8 and 16 km/h (Fishel and Ferrell 2010). Two of the three replicates of this study were conducted at 3.2 km/h and the low number of droplets on Teflon slides may indicate a spray drift, although there was a significant difference in mortality between control and treatment groups. Insecticide resistance status of this strain of *Ae. aegypti* is not known and unfortunately the study did not compare the effect of ULV application between a susceptible strain and the test strain. Such a comparison using CDC bottle bioassay would have provided information to ascertain whether the low mortality rates are due to acquired insecticide resistance. However, these results clearly show significant differences in mortality between control and treatment mosquitoes of both irradiated and non-irradiated groups. Further studies need to be conducted at optimal environmental conditions, especially at higher wind speeds, allowing for optimal downwind insecticidal spread to better characterize the influence of the ULV application and with a pyrethroid susceptible strain of *Ae. aegypti* to compare the effect with the test strain. As this is a semi-field experiment conducted with laboratory-reared mosquitoes, large scale field studies with released irradiated and wild males would be a supplement to the findings.

We believe that this is the first study to evaluate the impact of insecticide ULV spray on irradiated *Ae. aegypti*. Our results provide the first scientific evidence to support the commonly accepted belief that simultaneous use of SIT and ULV control strategies are not compatible for the control of *Ae. aegypti* populations, hence, SIT would be well suited toward the end of a IVM program to target the last remaining individuals of a population. This information will be of value in planning IVM programs that wish to incorporate SIT and adulticiding spray operations.

ACKNOWLEDGEMENT

The authors appreciate the support provided throughout the study by C. Efstathion, M. Gaines, D. Autry, M. Pearson, L. Bangonan, J. Dilla, J. Wynn, and F. Allen at Anastasia Mosquito Control District. Special thanks to W. Qualls for review of the manuscript.

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DIFFERENTIAL TOXICITY OF PYRETHROID AND ORGANOPHOSPHATE INSECTICIDES TO THE HONEY BEE, *APIS MELLIFERA* AND THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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Subject Editor: Keira Lucas

ABSTRACT

Six insecticide active ingredients (AIs) and five commercial insecticide formulations were applied by topical application and onto filter paper strips to determine differential toxicity to *Aedes aegypti* (L.) and *Apis mellifera* (L.), and to evaluate their potential use in future insecticide resistance monitoring surveys. For topical application, 0.1 or 1 µl of the technical insecticide solution was applied to the *Ae. aegypti* and *A. mellifera* thorax, respectively. For insecticide-impregnated strips the insecticide amount varied, according with the commercial formulation. By topical application deltamethrin was the most toxic AI (LD_{50} = 0.057 µg/g) to *Ae. aegypti* and prallethrin was least toxic (LD_{50} = 19.42 µg/g). For *A. mellifera*, the most toxic AIs were deltamethrin (LD_{50} = 0.013 µg/g) and bifenthrin (LD_{50} = 0.156 µg/g); and the least toxic was chlorpyrifos (LD_{50} = 3.246 µg/g). When the insecticide-impregnated papers method was used, Mosquitomist Two (chlorpyrifos 24.6%) was the most toxic insecticide for *Ae. aegypti* (LC_{50} = 0.024 µg/cm^2), and Aqualuer (permethrin 20.6%, PBO 20.6%) was least toxic (LC_{50} = 0.408 µg/cm^2). For *A. mellifera* the most toxic commercial insecticide formulations were Talstar (bifenthrin 7.9%; LC_{50} = 0.288 µg/cm^2) and Mosquitomist Two (LC_{50} = 0.299 µg/cm^2), with no significant differences, and the least toxic commercial formulation was Deltagard (deltamethrin 2.0%; LC_{50} = 15.084 µg/cm^2). By topical application, more than 28 times of chlorpyrifos was needed to obtain the same mortality in *A. mellifera* as in *Ae. aegypti*. When using the insecticide-impregnated paper method, more than 206 times of Deltagard was needed to obtain the same mortality in *A. mellifera* as in *Ae. aegypti*. Even though Mosquitomist Two was the most toxic insecticide for both insect species, the honey bees were >12 times more tolerant to this insecticide, compared with the mosquitoes.

Key words: *Aedes aegypti*, *Apis mellifera*, insecticides, toxicity, topical application, insecticide-impregnated papers, mosquito control

INTRODUCTION

Aedes aegypti (L.), the yellow fever mosquito, is an important vector of numerous human arboviral diseases including dengue, Zika, chikungunya and yellow fevers (CDC 2020a, 2020 b, 2020c, 2020d). Dengue, Chikungunya, and Yellow fever viruses may cause long-lasting severe symptoms and death. The illness caused by Zika virus is usually mild but may cause serious brain defects including microcephaly in unborn babies. Local transmissions of dengue and Zika have been reported from several states in the United States, including Florida. The Chikungunya and Yellow fever viruses are not currently present in the United States, but the risk of (re)introduction is possible due to infected travelers and the presence of *Ae. aegypti* (FDOH 2020a, 2020b).

About two-thirds of the crops traded on the world market depend on pollinator services (Klein et al. 2007). Honey bees, *Apis mellifera* (L.), are the most valuable pollinators for agricultural crops and the elevated loss rates of managed honey bee colonies

threaten the pollination services they provide (Bruckner et al. 2018, Klein et at. 2007, López-Uribe and Simone-Finstrom 2019). For that reason, there is global concern about the decline of honey bee 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 worker honey bees can forage up to 12 km around their hive and reach urban areas (Beekman and Ratnieks 2000), they can be exposed to insecticides used in public health to manage mosquitoes. *Ae. aegypti* is closely associated with urban and suburban domestic habits (Jansen and Beebe 2010), and insecticides are regularly applied to control them (Farook et al. 2018). Some studies concluded that barrier or ground insecticide applications to control host-seeking mosquitoes may also affect nontarget insects such as honey bees (Qualls et al. 2010, Drake et al. 2016). Adding to the challenges faced by mosquito control districts, *Ae. aegypti* is becoming increasingly resistant to pyrethroids (Smith et al. 2016, Estep et al. 2018 Casey et al. 2020), which are the active ingredients (AIs) of choice in many adulticides available for mosquito control. As such, novel ways are needed to control mosquitoes with minimal impacts on non-target organisms.

The first objective of the studies presented here was to determine the differential toxicity of one organophosphate and five pyrethroid AIs and one organophosphate and four pyrethroid commercial insecticide formulations for *Ae. aegypti* and *A. mellifera*. The second objective was to evaluate two bioassay methods for potential use in insecticide resistance monitoring surveys. This information is needed to help evaluate the impact of insecticide applications on both *Ae. aegypti* and *A. mellifera*.

MATERIALS AND METHODS

Insects rearing and maintenance. Pyrethroid-susceptible *Ae. aegypti* (ORL1952 strain) pupae in 473 ml (16 oz) deli cups were obtained from colonies maintained at the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA CMAVE) in Gainesville, FL, USA. Pupae and emerging adults were maintained in adult colony cages in an environmental chamber at 26+2°C (79+3°F), 50-80% RH and a photoperiod of 12:12 (Light:Dark). *Apis mellifera* were obtained from an apiary managed according to common practices for North Central Florida by the Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA. Female adult *Ae. aegypti* were collected 3-4 days after they had emerged from the pupal stage and used for the insecticide assays. Female adult worker *A. mellifera* were at least 3 days old and collected from three separate hives by shaking off adults crawling on hive frames. Throughout the experiments, adult *Ae. aegypti* and *A. mellifera* were provided with 10% and 50% sucrose solution *ad libitum,* respectively.

Active ingredient experiments. The following six commonly used mosquito adulticidal technical AIs (Sigma-Aldrich, USA) were used in the experiments: Phenothrin (94.6 %), prallethrin (96 %), deltamethrin (99.7%), chlorpyrifos (99.3%), permethrin (96.7%) and bifenthrin (99.1%). For range-finding experiments, 10-fold serial dilutions in acetone from $1.0x10^{4}$ - $1.0x10^{-1}$ ng/µL were applied topically onto the thorax of adult female *Ae. aegypti* and *A. mellifera*. Intermediate dilutions were included for the determination of the LD_{50}.

For *Ae. aegypti*, topical toxicity bioassays were performed based on the method of Pridgeon et al. (2008). For each of 5 replicate assays per treatment, 10 adult female *Ae. aegypti* were knocked down using CO_2 for 15 s, and then treated with 0.1 µl of insecticide preparation using a 5 µl syringe (Hamilton Co. Reno NV) with a repeat dispenser (Hamilton PB 600-1). Each group of treated mosquitoes were transferred to a 20-ml scintillation vial, which was covered with mesh to prevent escape. Control insects were treated with acetone only. Mortality was assessed 24 h after exposure to insecticides. The replicates were performed on different days with

4-5 doses over the critical portion of the dose curve for Probit analysis.

For *A. mellifera*, topical toxicity bioassays were performed based on the method of the Organization for Economic Co-operation and Development (OECD 1998). For each of 6-7 replicate assays per treatment, 10 adult female workers from three separate hives were knocked down with CO_2 for 20 s and then treated with 1.0 µl of insecticide preparation using a 50 µl syringe (Hamilton Co. Reno NV) with a repeat dispenser (Hamilton PB 600-1). Each group of treated insects were transferred to a 120-ml Mason jar which was then closed with a lid that was modified with glued-in mesh. Negative controls were treated with acetone only. Mortality was assessed 24 h after exposure to insecticides. The replicates were performed on different days with 4-5 doses over the critical portion of the dose curve for Probit analysis.

Commercial insecticide experiments. The following five commercial insecticides were tested using an insecticide-impregnated paper method: Mosquitomist Two™ (chlorpyrifos 24.6%; Clarke Roselle, IL), Aqualuer® 20-20 (permethrin 20.6%, PBO 20.6%; AllPro Vector Group, St Joseph, MO), Deltagard® (deltamethrin 2.0%; Bayer Cropscience, Cary, NC), Duet® (Prallethrin 1.0%, Phenothrin 5.0%, PBO 5.0%; Clarke, Roselle, IL) and Talstar P (Bifenthrin 7.9%; FMC, Philadelphia PA). For range-finding experiments, 10-fold serial dilutions from $1.0x10^{0}$ - $1.0x10^{-5}$ % were prepared using different diluents depending on the miscibility of the pesticide formulation. Mosquitomist Two and Aqualuer were diluted in acetone; Deltagard and Talstar in distilled water; and Duet in mineral oil. Intermediate dilutions were included for the determination of the LC_{50}.

Each insecticide preparation was applied to filter paper strips (Whatman filter paper #2). For *A. mellifera*, the strips were 14 cm^2 (2x7 cm), and for *Ae. aegypti* the strips were 5 cm^2 (1x5 cm). To ensure the same amount of AI/cm^2, the volume of insecticide solution applied was adjusted based on the size of the paper strip and the solvent used. *A. mellifera* strips were treated with 90 µl of Mosquitomist Two or Aqualuer preparations; 140 µl of Deltagard or Talstar preparations, or 70 µl of Duet preparation. *Ae. aegypti* strips were treated with 32 µl of Mosquitomist Two or Aqualuer, 50 µl of Deltagard or Talstar, or 25 µl of Duet preparations (Sanchez-Arroyo et al., 2019). The negative control strips were treated with the diluents of the corresponding insecticides.

Aedes aegypti were knocked down using CO_2 for 15 s and transferred to 20-ml scintillation vials, which were then covered with mesh secured by rubber bands. Ten females were used in each concentration replicate and housed in the same vial. After 30 minutes and complete insect recovery from CO_2, an insecticide-treated filter paper strip was introduced to the middle of the scintillation vial, with both sides available for mosquitoes to rest. The strips remained in the vial for the duration of the experiment. Five replicates were carried out on separated days.

Apis mellifera were knocked down using CO_2 for 20 s and transferred to 120-ml glass jars which were then secured with a mesh. Ten worker bees were used in each concentration replicate and housed in the same jar. After 30 minutes and complete recovery from CO_2, a filter paper strip treated with insecticide was introduced to the center of the jar with both sides exposed. The strip remained in the jar for the duration of the experiment. Any bees that were not walking at the time the insecticide-treated paper strip was added, were not included in the experiment. Five to seven replicates were carried out on separate days.

At least 350 *Ae. aegypti* or *A. mellifera* were assayed against each insecticide. For both insects, mortality was assessed 24 h after exposure to insecticides.

Statistical Analysis. To determine the LD_{50} and LC_{50} for each AI and insecticide formulation, respectively, a probit analysis was performed with SAS version 9.4 (SAS Institute Inc., Cary, NC), and significance was determined by non-overlap of 95% confidence limits. If negative control mortality was >5% , mortality data of the corresponding treatments were corrected with Abbott's formula (Abbott 1925).

RESULTS

Toxicity of the active ingredients by topical application. For *Ae. aegypti*, deltamethrin was the most toxic of the 6 AIs, followed by bifenthrin, chlorpyriphos, phenothrin, permethrin, and prallethrin (Table 1, Table 3). For *A. mellifera,* the most toxic AI was deltamethrin, followed by bifenthrin, permethrin, phenothrin, prallethrin, and chlorpyriphos which was the least toxic AI (Table 1, Table 3). The honey bee tolerance index was largest for chlorpyriphos (28.72), followed by phenothrin (11.44), permethrin (3.95), bifenthrin (2.64), deltamethrin (0.228), and prallethrin (0.14) (Table 1). This means that much more chlorpyriphos was needed to kill *A. mellifera* than susceptible *Ae. aegypti* but, conversely, much less prallethrin or deltamethrin. Phenothrin, permethrin, and bifenthrin were moderately to slightly less toxic to *A. mellifera* than to *Ae. aegypti.*

Toxicity of insecticide formulations by paper bioassay. When the insecticide-impregnated papers method was used, Mosquitomist Two was most toxic to *Ae. aegypti*, followed by Talstar, Duet, Deltagard, and Aqualuer (Table 2, Table 3). For *A. mellifera*, the most toxic commercial insecticide formulation was Talstar, followed by Mosquitomist Two , Duet, Aqualuer, and Deltagard (Table 2, Table 3). The honey bee tolerance indexes show that *A. mellifera* was more tolerant than *Ae. aegypti* to all five insecticide formulations, with Deltagard being the least toxic, and Talstar the most toxic (Table 2). This means, for example, that > 200 times of Deltagard was needed to kill *A. mellifera* than susceptible *Ae. aegypti.*

Insects were not only observed for mortality 24 h post treatment, but behavior was also assessed during the time of exposure. Both insect species behaved differently when exposed to the pyrethroids as opposed to the organophosphate formulation. The insects walked for only shorts periods of time on the pyrethroid-impregnated papers; apparently trying to avoid them. This behavior was not observed when the insects were exposed to chlorpyrifos.

Table 1. Lethal doses (LD_{50}) of six insecticides topically applied to *Aedes aegypti* and *Apis mellifera* adults.

	Aedes aegypti			*Apis mellifera*			
Insecticide	LD_{50} (µg/g insect)	(95% CI)	Slope (SE)	LD_{50} (µg/g insect)	95% CI	Slope (SE)	Honey Bee Tolerance Index[1]
Deltamethrin*	0.057a	0.050-0.067	1.88 (0.149)	0.013a	0.008-0.018	2.27 (0.39)	0.228
Bifenthrin*	0.059a	0.051-0.069	2.10 (0.183)	0.156b	0.137-0.177	2.71 (0.24)	2.64
Chlorpyrifos*	0.113b	0.105-0.158	1.55 (0.169)	3.246d	2.902-3.622	3.52 (0.35)	28.72
Phenothrin*	0.168bc	0.150-0.187	3.25 (0.307)	1.922c	1.703-2.160	2.08 (0.23)	11.44
Permethrin*	0.194c	0.170-0.221	2.63 (0.28)	0.767b	0.0674-0.887	2.66 (0.25)	3.95
Prallethrin*	19.42d	17.53-21.51	3.55 (0.32)	2.603d	2.290-2.950	3.56 (0.35)	0.134

[1]*A. mellifera* LD_{50} / *Ae. aegypti* LD_{50}

*Means followed by the same letter within a treatment group for each species are not significantly different.

Table 2. Lethal concentrations (LC_{50}) of commercial insecticides to *Aedes aegypti* and *Apis mellifera* adults, using insecticide-impregnated paper method.

	Aedes aegypti				*Apis mellifera*				
Insecticide	LC_{50} (µg/cm²)	95% CI	Slope (SE)	N	LC_{50} (µg/cm²)	95% CI	Slope (SE)	N	Honey Bee Tolerance Index[1]
Deltagard* (AI deltamethrin)	0.073a	0.059-0.091	1.65 (0.160)	360	15.084a	9.37-23.66	2.29 (0.426)	490	206.6
Talstar* (AI bifenthrin)	0.030b	0.026-0.035	2.44 (0.214)	360	0.288b	0.259-0.323	3.26 (0.314)	350	9.6
Aqualuer* (a.i permethrin)	0.408c	0.363-0.461	3.142 (0.282)	360	4.647c	2.65-9.249	1.168 (0.175)	490	11.39
Mosquitomist Two* (AI chlorpyrifos)	0.024b	0.021-0.027	3.06 (0.320)	360	0.299b	0.267-0.333	3.04 (0.250)	420	12.45
Duet* (AIs sumithrin and prallethrin)	0.069a	0.054-0.070	2.59 (0.247)	460	2.302d	2.043-2.627	2.75 (0.245)	490	33.36

[1]*A. mellifera* LC_{50} / *Ae. aegypti* LC_{50}

*Means followed by the same letter within a treatment group for each species are not significantly different.

Table 3. Comparative Toxicity of Active Ingredients Tested by Topical Application and Surface Treatment.

	Ae. aegypti		*A. mellifera*	
	Topical Application	Filter paper strips	Topical Application	Filter Paper Strips
Most toxic	Deltamethrin (a)	Mosquitomist Two (a) (AI chlorpyriphos)	Deltamethrin (a)	Talstar (a) (AI bifenthrin)
	Bifenthrin (a)	Talstar (a) (AI bifenthrin)	Bifenthrin (b)	Mosquitomist Two (a) (AI chlorpyriphos)
↓	Chlorpyriphos (b)	Duet (AIs sumithrin, prallethrin) (b)	Permethrin (b)	Duet (AIs sumithrin, prallethrin) (b)
	Phenothrin (c)	Deltagard (AI deltamethrin) (b)	Phenothrin (c)	Aqualuer (AI permethrin) (c)
Least toxic	Permethrin (c)	Aqualuer (AI permethrin) (c)	Prallethrin (d)	Deltagard (AI deltamethrin) (d)
	Prallethrin (d)		Chlorpyriphos (d)	

*Means followed by the same letter within a treatment group for each species are not significantly different.

DISCUSSION

The pyrethroid AIs deltamethrin and bifenthrin were most toxic to both *Ae. aegypti* and *A. mellifera*, when applied topically. Bifenthrin was also most toxic when the insects were exposed to treated filter paper strips, but deltamethrin was much less toxic (Table 3). Instead, the organophosphate, chlorpyrifos, was very toxic to both insect species when exposed to treated filter paper strips. Chlorpyrifos had an intermediate insecticide toxicity for *Ae. aegypti* and was least toxic for *A. mellifera* when applied topically (Table 3).

In the topical application method, immobilized insects are treated with insecticide, and the doses are independent of insect activity (Moses and Gfeller 2001). In the insecticide-impregnated method, insects are actively exposing themselves to insecticide when walking on the treated strips, and the amount of insecticide picked up is a function of time spent on the treated surface. For *Ae. aegypti* and both methods of insecticide application, < 1 µg of active ingredient or formulation/g insect resulted in 50% mortality, with the notable exception of prallethrin. For *A. mellifera*, insecticide-impregnated paper strips tended to be less toxic than topically applied insecticides, with the notable exception of Mosquitomist Two (Tables 1 and 2). One possible reason could be that the insects walked for longer periods of time on the chlorpyrifos-treated papers than on the pyrethroid-treated papers, and hence picked up more chlorpyriphos AI by tarsal contact. This may, in part, explain why chlorpyrifos was more toxic than three of the four pyrethroid insecticides. Danka et al. (1986) also suggested that insecticide cuticular penetration in honey bees is slower for applications made to the thorax than tarsi due to differences in sclerotization in those areas. On the other hand, when summarizing the toxicity data of insecticides, Hardstone and Scott (2010) reported that while honey bees can be sensitive to individual insecticides, they are not highly sensitive to insecticides overall, or even to specific classes of insecticides.

There are few reports in the literature on the toxicity of modern insecticides to honey bees. Greig-Smith et al. (1994) reported LD_{50} of 0.59 µg/g bee for chlorpyrifos, and Hardstone and Scott (2010) an LD_{50} range from 0.590 to 1.14 µg/g bee for the same insecticide. In this research we reported an LD_{50} of 3.24 µg/g for honey bees. For permethrin, Inglesfield (1989) reported an LD_{50} of 1 µg/g bee, meanwhile Danka (1986) reported an LD_{50} of 0.15 µg/g bee. In our study we obtained an LD_{50} of 0.767 µg/g bee, an intermediate value.

Topical application is a method where the insecticide is deposited directly onto the insect thorax, and allows the development of defined toxicological data for calculation of resistance ratios, a measure that World Health Organization (WHO 2009, 2018) and CDC bioassays were not designed to produce (Waits et al. 2017). This data is useful in comparing topical application with Ultra Low Volume (ULV) application, either using truck-mounted equipment or any kind of aircraft (Mount et al. 1996), since the droplets directly impinge the insect body. In the present study, the only difference is the insect size, since the honey bees are about 20 times bigger than mosquitoes.

The insecticide-impregnated papers method was originally developed to evaluate discriminating doses. In this method, the insects expose themselves to the insecticide; the more they move, 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). Insecticide contact with appendages such as the leg resulted in much lower mortality from both permethrin and malathion and suggest that topical bioassay techniques used to evaluate mortality to *Culex quinquefasciatus* (Say) may be modified to include other body areas without reducing comparability to mesothorax studies (Aldridge et al. 2016). Insecticide toxicity determined by exposure to insecticide-impregnated filter paper is useful for comparison with barrier treatments, since in this type of operational insecticide application (either using a backpack sprayer or a

truck mounted-mist sprayer) we expect the insect to pick up the lethal amount of insecticide by their tarsi (VanDusen et al. 2016, Richards et al. 2017).

Irritation produced by pyrethroid insecticides may have prevented the insects from staying in contact with the insecticide-impregnated papers for a longer time. Since chlorpyrifos did not cause irritation, this may explain why it is more toxic to both insects, because they move freely or rest on insecticide-impregnated papers until they get a lethal dose. This toxicity may not be correlated with the insect's body weight (Robertson et al. 2017). From a practical point of view, it could be more useful to use insecticide-impregnated papers rather than topical treatments in order of generate more useful information about field insecticide effects on these species. The exposure to insecticide-impregnated papers has been proposed to carry out toxicological studies for monitoring of *Triatoma infestans* populations, and other insects (Remón et al. 2017).

Atkins et al. (1973, 1975; cited by Danka et al. 1986) reported that most referenced insecticide results are topical or contact, and the LD_{50} concentrations obtained by topical application are relatively lower. Felton et al. (1986) suggested that the data on the acute contact and oral toxicity of pesticides to honey bees should be expressed as LD_{50} and should be considered as one of the elements for assessment of danger to foraging honey bees. However, the current study provides evidence that the insecticide-impregnated paper method has value in determining which residual insecticides have the least effect on field nontarget species such as honey bees. Since commercial formulations were used in the insecticide-impregnated method, the results could provide guidance on which insecticides to use in the field. This information is needed to eliminate, as much as possible, non-target effects on honey bees which have comparatively few genes encoding detoxification enzymes (Claudianos et al. 2006).

Pyrethroids are the most common insecticides used for adult mosquito control, which has led to widespread resistance globally. Resistance to permethrin and other pyrethroids in mosquitoes were recently documented in Florida (Coleman et al. 2017, Estep et al. 2018, Parker et al. 2020). Honey bees are moderately sensitive to deltamethrin and permethrin, and more sensitive to bifenthrin (Hardstone and Scott 2010), and the application of these insecticides when pollinators are not foraging to avoid mortality of honey bee and other non-target insects becomes even more important when targeting pyrethroid-resistant mosquitoes. Correct application timing combined with better insecticide application techniques can further increase safety of mosquitocidal applications. Aerial ultra-low volume applications using high-pressure nozzle system reduced environmental insecticide contamination with Naled and leads 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 honey bees and other non-target insects.

ACKNOWLEDGMENTS

We thank Drs. Jamie Ellis and Branden Stanford (both from Entomology and Nematology Department, University of Florida) for supplying the honey bees, and the USDA CMAVE for supplying the *Aedes aegypti*. 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.

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EFFECT OF COPPER SULPHATE PENTAHYDRATE ON MOSQUITO LARVAL *AEDES AEGYPTI*, *CULEX QUINQUEFASCIATUS*, AND *ANOPHELES QUADRIMACULATUS* IN LABORATORY AND UNDER SEMI-FIELD CONDITIONS

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Guest Editor: Vindhya S. Aryaprema

ABSTRACT

Mosquito larval control has been conducted by various chemicals and biological agents to reduce mosquito population and mosquito-borne diseases. The larvicidal efficacy of Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) on *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles quadrimaculatus* was evaluated separately in the laboratory and semi-field conditions. Different concentrations of $CuSO_4 \cdot 5H_2O$ (ranging from 1 to 20 ppm) were tested against third (3^{rd}) instar larvae. Larval mortality was observed at 24, 48 & 72h after exposure and the LC_{50} values were determined. In both conditions, larval mortality showed concentration and time dependent correlations i.e. larval mortality was higher with increasing concentration $CuSO_4 \cdot 5H_2O$ and exposure time. No mortality was observed in the control (0 ppm). Of the three species tested, *Cx. quinquefasciatus* and *An. quadrimaculatus* were more sensitive to $CuSO_4 \cdot 5H_2O$ than *Ae. aegypti*. It was demonstrated that 1.5 -2.25 ppm of $CuSO_4 \cdot 5H_2O$ killed more than 50% of *Cx. quinquefasciatus* and *An. quadrimaculatus* larvae at 72 h in both laboratory and semi-field conditions, whereas *Ae. aegypti* could survive easily in these concentrations. Besides, $CuSO_4 \cdot 5H_2O$ showed more toxicity to larvae in semi-field conditions than laboratory studies. These results suggest that $CuSO_4 \cdot 5H_2O$ could be used as a potential larvicide especially for *Cx. quinquefasciatus* and *An. quadrimaculatus* as a low-cost alternative larvicidal agent. Further studies will be needed to confirm its effectiveness in large scale field trials.

Key Words: Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), *Ae. aegypti*, *An. quadrimaculatus*, *Cx. quinquefasciatus*, larvicide

INTRODUCTION

Mosquitoes are one of the deadliest organisms in the world and a serious threat to public health. Mosquito-borne diseases are prevalent in more than 100 countries across the world that causes millions of deaths every year (WHO, 2009). More than half of the world's population live in the areas with a risk of mosquito-borne diseases. Diseases such as dengue, chikungunya, yellow fever, Zika, malaria, filariasis, Japanese encephalitis, West Nile fever, etc. are transmitted among humans mainly by three genera of mosquitoes, *Aedes, Anopheles* and *Culex* (Remia and Logaswamy, 2010; Arivoli et al., 2011). Therefore, mosquitoes and mosquito-borne diseases have become challenging problems that have social and economic impacts (Raveen et al., 2014).

Adult and larval mosquito control have been undertaken by many vector control programs to suppress mosquito-borne diseases in many countries. Larval control has been assumed as the main strategy for successful mosquito control programs. Chemical insecticides are commonly considered to be the most effective control strategy against mosquitoes. However, concern has increased significantly regarding their negative effects, such as the development of resistance in mosquitoes, toxicity to non-

target organisms, potential health hazards, water contamination, environmental pollution, and residual effects (Ndakidemi et al., 2016). In recent years, *Bacillus thuringiensis israelensis* (Bti) and insect growth regulators (IGRs) have been used widely to control mosquito larvae. These are comparatively expensive, and they have some limitations too. Scientists, therefore, have been looking for alternatives for managing the mosquito larvae. Accordingly, we attempted in this study to utilize Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) solution as a mosquito larvicide because it is a cheap alternative to Bti and IGRs. $CuSO_4 \cdot 5H_2O$ is easily obtained and maintained, and has bactericidal, algicidal and fungicidal effects, which is beneficial for bacterial, algal or fungal contamination (Biagi et al., 2014).

Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), commercially formulated as REXCU-S (DNW Global, USA), has been used for the suppression of bacterial odors and toxic gas in standing or moving water bodies containing organic matter of algae or bacteria. It is used worldwide as an algaecide and a fungicide in aquaculture and agriculture (Lasiene et al., 2016). Elevated levels of copper in water is long known to adversely affect survival, growth, reproduction, feeding and even cause morphological deformity on aquatic organisms (Hodson et al., 1979). It is also used as a therapeutic chemical for various ectoparasitic and bacterial infections. The toxic effect of $CuSO_4 \cdot 5H_2O$ was examined in freshwater fish, *Capoeta umbla* (Kirici et al., 2017). The acute and chronic toxicity of copper to aquatic midge like *Chironomus ramosus* (Majumdar and Gupta 2012), *Chironomus tentans* (Nebeker et. al., 1984 and Warrin et. al., 2009) and *Chironomus decorus* (Kosalwat and Knight 1987) was studied previously to determine the LC_{50} values. The ability of copper to kill or injure mosquito larvae was studied by Reza et al. (2012). Their study revealed that copper, in both its solid and liquid forms, was lethal to mosquito larvae at a concentration of 1.2 ppm (Reza et al., 2012). Furthermore, in another study in 2014, they demonstrated that 10 ppm of copper solution could kill more than 90% larvae of *An. stephensi, Ae. albopictus* and *Cx. pipiens pallens* at 96 hours exposure (Reza et al., 2014). These results encouraged us to evaluate $CuSO_4 \cdot 5H_2O$ solution as a mosquito larvicide.

The present study investigated the larvicidal potential of $CuSO_4 \cdot 5H_2O$ against 3rd instar larvae of *Ae. aegypti*, *An. quadrimaculatus,* and *Cx. quinquefasciatus* under laboratory and semi-field conditions.

MATERIALS AND METHODS

Mosquito larvae. Three species of mosquitoes *Ae. aegypti*, *An. quadrimaculatus* and *Cx. quinquefasciatus* were maintained at the insectary, Anastasia Mosquito Control District (AMCD), Florida, USA. The eggs of mosquitoes (target species) were hatched and kept in water up to 3rd instar larvae. The third (3rd) instar larvae of each species were collected from the insectary and used in this study to conduct trials in the laboratory and semi field conditions.

Larvicide. A commercial solution of $CuSO_4 \cdot 5H_2O$ (REXCU-S) [(19.8 % active ingredient-a.i. and 80.2 % other ingredients), a soluble liquid blue in color] was purchased from DNW Global LLC, Florida, USA. For larval bioassay, the concentrations (ppm) were prepared on the a.i. of $CuSO_4 \cdot 5H_2O$.

Larval bioassays. Bioassays were carried out separately in the laboratory and semi-field conditions for all species of mosquito larvae. The 3rd instar larvae were visually detected using size as the determinant. Larvicidal activity (percentage of mortality) and LC_{50} values were calculated using the WHO (2005) bioassay protocol with slight modifications. The tested larvae were free from any exposure to insecticides or chemicals.

In the laboratory conditions, seven (7) different concentrations (1, 2, 6, 10, 14, 18, and 20 ppm) of $CuSO_4 \cdot 5H_2O$ (a.i.) were prepared from the stock solution using distilled water (stock solution is made just before experimentation). Ten larvae of each species were released by means of a dropper into a 250 mL transparent plastic cup containing 100 mL of each concentration of the solution. A control (distilled water only) was also included with each concentration. For each

concentration, four (4) replicates were conducted to check the mortality in a completely randomized design (CRD). All the experimental cups were placed on a tray and kept it in the incubator at 26 (2±) ˚C under 12:12 light: dark regime with 80% relative humidity. No food was provided for larvae during this experimentation.

In the semi-field conditions, five (5) different concentrations (2, 5, 10, 15 and 20 ppm) of $CuSO_4 \cdot 5H_2O$ (a.i.) were prepared from the stock solution using distilled water. For *Ae. aegypti,* twenty-five (25) larvae were released by means of a dropper into a 20 L black plastic bucket containing 9 L of each concentration of the solution. A control (distilled water only) was also included with each concentration. For each concentration, four (4) replicates were conducted to check the mortality in a CRD. All the experimental buckets were kept in the AMCD field (location: 29°54'09.0"N 81°24'46.4"W) under natural environment. No food was provided for larvae during this experimentation. For *An. quadrimaculatus* and *Cx. quinquefasciatus,* two hundred (200) larvae were released by means of a dropper into a 950 L cement tank containing 750 L of each concentration of the solution made with well water. A control (well water only) tank was also maintained for each concentration. Four (4) replicates were conducted for each concentration to check the mortality. All the experimental tanks were in the AMCD field (location: 29°54'08.4"N 81°24'47.1"W) under natural environment. A little food (2 grams fish food) was provided for larvae during the experimentation.

Larval mortality for each condition were determined by counting the number of dead larvae. Larvae were considered dead if they showed no sign of movement even after being touched with a glass rod (Langat et al., 2012). The percentage of larval mortality was recorded after 24, 48, and 72 h and corrected using Abbott's formula (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{\text{\% mortality in treated} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \wedge 100$$

Statistical analysis. Statistical analysis of the experimental data was performed with "MS EXCEL 2010 program" and GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA) to find out mortality percentage, regression equations (y), and correlation coefficient values (r). The LC_{50} values were estimated using Probit analysis (Finney, 1971).

RESULTS

Effect of $CuSO_4 \cdot 5H_2O$ in Laboratory conditions.

The percentages of larval mortality at 24, 48, and 72 h after exposure to the seven (7) different concentrations of the $CuSO_4 \cdot 5H_2O$ solution are presented in Table 1. Mortalities increased with an increase in the concentration (ppm) of $CuSO_4 \cdot 5H_2O$ solution for all species at any time period of exposure during experiment. At 72 h exposure time, the highest mortality (97.5 to 100%) was observed for all species. The correlation analysis showed that mortality and concentration was positively correlated (r) for all species (Fig. 1).

The LC_{50} values of $CuSO_4 \cdot 5H_2O$ solution at 24, 48, and 72 h after exposure against the 3rd instar larvae of *Ae. aegypti, An. quadrimaculatus,* and *Cx. quinquefasciatus* were determined (Table 3). The LC_{50} values decreased with the increase of larval exposure time. The $CuSO_4 \cdot 5H_2O$ solution exhibited the highest larvicidal activity at 72 h after exposure against *Ae. aegypti, An. quadrimaculatus,* and *Cx. quinquefasciatus* larvae with their lowest LC_{50} values of 5.5, 2.25 and 2 ppm, respectively.

Effect of $CuSO_4 \cdot 5H_2O$ in Semi-field conditions.

The percent larval mortality at 24, 48, and 72 h after exposure to the five (5) different concentrations of the $CuSO_4 \cdot 5H_2O$ solution are presented in Table 2. Mortalities increased with the increase of the concentration of the $CuSO_4 \cdot 5H_2O$ solution for all species at any time period of exposure. At 48 h exposure time, the highest mortality (100%) was observed for all species. The correlation

Table 1. Larval mortality (%) of *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus* at 24, 48, and 72 h after exposure to CuSO4·5H2O solution (REXCU-S) in laboratory conditions.

		% Mortality (Mean)		
Mosquito species	Concentration (ppm)	24 h	48 h	72 h
Ae. aegypti	0 (Control)	0	0	0
	1	0	5	5
	2	5	10	17.5
	6	12.5	45	57.5
	10	30	57.5	65
	14	52.5	82.5	82.5
	18	77.5	92.5	95
	20	87.5	97.5	97.5
An. quadrimaculatus	0 (Control)	0	3	4
	1	7.5	20	35
	2	10	25	50
	6	25	37.5	87.5
	10	42.5	62.5	82.5
	14	67.5	82.5	92.5
	18	77.5	80	97.5
	20	92.5	92.5	100
Cx. quinquefasciatus	0 (Control)	0	0	0
	1	0	5	42.5
	2	7.5	17.5	57.5
	6	25	42.5	95
	10	25	67.5	95
	14	42.5	95	100
	18	75	92.5	97.5
	20	75	95	100

analysis showed that mortality and concentration is positively correlated (r) for all species (Fig. 1). This gradient of positive dependency between mortality and concentration is the key and common characteristic of any functional larvicide.

The LC_{50} values of the $CuSO_4{\cdot}5H_2O$ solution at 24, 48, and 72 h after exposure against the larvae of *Ae. aegypti*, *An. quadrimaculatus,* and *Cx. quinquefasciatus* were determined (Table 3). The LC_{50} values decreased with the increase of larval exposure time. The $CuSO_4{\cdot}5H_2O$ solution exhibited the highest larvicidal activity at 72 h after exposure against *Ae. aegypti*, *An. quadrimaculatus,* and *Cx. quinquefasciatus* larvae with the lowest LC_{50} values of 3, 1.5 and 1.5 ppm, respectively.

Significant differences were observed in the efficacy of the $CuSO_4{\cdot}5H_2O$ solution to kill larvae when evaluated in the laboratory and semi-field conditions. The LC_{50} values of $CuSO_4{\cdot}5H_2O$ against the larvae of *Ae. aegypti*, *An. quadrimaculatus,* and *Cx. quinquefasciatus* were comparatively low in semi-field conditions compared to laboratory assays. Altogether, in this study, the $CuSO_4{\cdot}5H_2O$ solution was observed with higher toxicity to *Cx. quinquefasciatus* and *An. quadrimaculatus* than to *Ae. aegypti* in both laboratory and semi-field studies.

DISCUSSION

The $CuSO_4{\cdot}5H_2O$ solution exhibited prominent larvicidal activity at different concentrations against 3rd instar larvae of *Ae. aegypti*, *An. quadrimaculatus* and *Cx. quinquefasciatus* during laboratory and semi-field studies.

This gradient of positive dependency between mortality and concentration revealed that $CuSO_4{\cdot}5H_2O$ could be a rational larvicide for mosquitoes. The high mortality rate

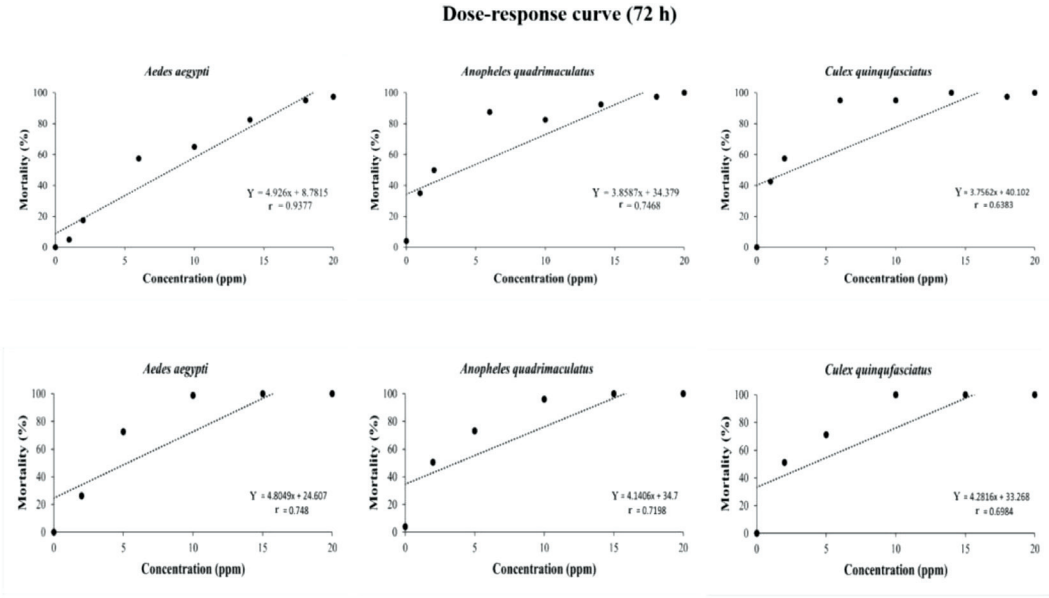

Figure 1. Dose-response curve (Linear regression equation (Y) and correlation coefficient (r) between CuSO4·5H2O concentration and larval mortality at 72 h after exposure). The mortality percentage increased with increasing concentrations showed a positive correlation.
(upper panel: lab condition; lower panel: semi-field condition)

may have been due to the large number of copper molecules that bound with receptors in the larval midgut, because copper was previously shown to damage the peritrophic matrix in the midgut (Beaty et al., 2002). In contrast, it is speculated that larval exposure to $CuSO_4{\cdot}5H_2O$ solution for a short period of time at a lower concentration resulted in a lower mortality rate because of a reduction in copper ions binding with the receptors in

Table 2. Larval mortality (%) of *Aedes aegypti*, *Anpheles quadrimaculatus*, and *Culex quinquefasicatus* at 24, 48, and 72 h after exposure to CuSO4·5H2O solution (REXCU-S) in semi-field conditions

		% Mortality (Mean)		
Mosquito species	Concentration (ppm)	24 h	48 h	72 h
Ae. aegypti	0 (Control)	0	0	0
	2	7.5	20	26.25
	5	41.25	66.25	72.5
	10	88.75	97.5	98.75
	15	96.25	100	100
	20	97.5	100	100
An. quadrimaculatus	0 (Control)	1.5	3	4
	2	9.38	15	50.38
	5	24.88	31.75	73.13
	10	78.75	86	96
	15	94.38	100	100
	20	100	100	100
Cx. quinquefasciatus	0 (Control)	0	0	0
	2	3.38	18.50	51
	5	67.63	76.38	71.25
	10	86	100	100
	15	90	100	100
	20	95.38	100	100

Table 3. Probit analysis of the larvicidal efficacy of CuSO4.5H2O solution (REXCU-S) against *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus* at 24, 48, and 72 h after exposure in laboratory and semi-field conditions.

	LC50 (ppm) of CuSO4.5H2O					
	Ae. aegypti		*An. quadrimaculatus*		*Cx. quinquefasciatus*	
Time duration (Hour)	Lab.	Semi-field	Lab.	Semi-field	Lab.	Semi-field
24	13	5	11	6.5	14.5	4.5
48	7	3.5	7.5	5	7	3
72	5.5	3	2.25	1.5	2	1.5

LC_{50}: Lethal concentration to kill 50% of the exposed larvae.

the larval midgut. However, further experiments may be needed to validate this.

Based on the LC_{50} values obtained from the laboratory evaluation, the order of susceptibleness among the larvae was as follows: *Cx. quinquefasciatus* (2 ppm) > *An. quadrimaculatus* (2.25 ppm) > *Ae. aegypti* (5.5 ppm) after 72h. This result is in accordance with a study reported by Reza et. al. (2014), that at 3.3 ppm concentration of copper solution, *Cx. pipiens* was more susceptible than *An. stephensi* and *Ae. albopictus*.

Based on the LC_{50} values obtained from the semi-field evaluation, the order of susceptibleness among the larvae was as follows: *Cx. quinqufasciatus* (1.5 ppm) = *An. quadrimaculatus* (1.5 ppm) > *Ae. aegypti* (3 ppm) at 72h. Interestingly the order of susceptibleness among the mosquito larvae was the same as our laboratory test which was also supported by Reza et al. 2014.

In monitoring mortality, the semi-field results showed maximum larval mortality within earlier exposure time of 24 and 48 h while in the laboratory it was prolonged up to 72 h. Besides, the observed LC_{50} values in semi-field conditions were comparatively lower than the laboratory conditions at any time period of exposure during experiment. The semi-field results might be attributed to different environmental factors like light, heat, etc. hence causing more larvicidal effect. Therefore, it can be said that $CuSO_4$ solution is more toxic to mosquito larvae in semi-field conditions, compared to laboratory conditions.

It is summed up that the tested $CuSO_4{\cdot}5H_2O$ have larvicidal activity against the mosquito larvae, especially on *Cx. quefaciatus* and *An. quadrimaculatus*. This easy, safe, and low-cost alternative larvicide may be recommended for mosquito larval control. Continuous use of insecticide for controlling mosquitos and their larvae can enhance the resistance population. Therefore, a low concentration of $CuSO_4{\cdot}5H_2O$ can be used as an alternative along with other chemical or biological insecticides for which problems have been reported with toxicities and resistances (Tetreau et al., 2012). This $CuSO_4{\cdot}5H_2O$ solution may be directly used at the breeding sites of mosquitoes in stagnant water and in localized conditions. Thus, the findings of this study have demonstrated that the mortality of larvae by $CuSO_4{\cdot}5H_2O$ is worth for further studies in open water bodies. Although several environmental issues are associated with copper, the careful utilization and strict control of low concentrations and limited usage will avoid contamination in the environment.

ACKNOWLEDGMENT

The authors would like to acknowledge to M.K. Gaines, R. Weaver, V. Aryaprema and other AMCD employees for their partial participation in this research study. The authors also extend their sincere appreciation to J. Davidson, DNW Global, LLC, USA for supporting this research work.

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INSECTICIDE EFFICACY OF SPATIAL REPELLENT COMPOUND-METOFLUTHRIN AGAINST SUSCEPTIBLE AND RESISTANT STRAINS OF *AEDES AEGYPTI*

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ABSTRACT

Spatial repellents (SR), include pyrethroid insecticides that are highly volatile at low temperatures and with high lethal activities against mosquitoes, mainly *Aedes* vectors of arboviral diseases. Of these SR, metofluthrin is widely used in various devices for repellent consumer products. This article reports the susceptibility status of *Ae. aegypti* Puerto Rico permethrin-resistant laboratory strain (PR) and Orlando susceptible laboratory strain (ORL) to metofluthrin and permethrin using the CDC glass bottle bioassay. The time-mortality relationships showed that the permethrin-resistant PR strain is highly resistant to both permethrin and metofluthrin compared to the susceptible ORL strain. The resistant ratio (RR) based on the killing time (KT) (KT_{50}-PR/KT_{50}-ORL) was 30- and 5- folds for permethrin and metofluthrin, respectively. The results also showed that the PR strain is less resistance to metofluthrin than to permethrin, with a three-fold RR (KT_{50}-PR-per/KT_{50}-PR-met). These results indicate the potential risk of developing cross-resistance of metofluthrin in permethrin-resistant mosquitoes. Integrated vector management in mosquito control should be considerate of how consumer products and field operations interact to accelerate cross resistance to pyrethroids.

Key Words: *Aedes aegypti*, resistance, metofluthrin, permethrin, spatial repellents

INTRODUCTION

Aedes aegypti (Linn.) is a major vector of arboviral disease causing pathogens, such as yellow fever, dengue, zika, and chikungunya viruses. Application of repellents is one technique for prevention and control of mosquitoes and mosquito-borne diseases. Spatial repellents (SR) include a class of volatile pyrethroids, such as transfluthrin and metofluthrin, which have high insecticidal activities (Bibbs et al. 2018), high volatility at low temperatures, and low mammalian toxicity (Sugano and Ishiwatari 2012). These features facilitated their use in different commercial mosquito repellent devices (coils, liquid, cold fan vaporizers, paper and resins-based emanators) against various mosquito vectors, mainly, *Ae. aegypti* and *Ae. albopictus* Skuse (Ujihara et al. 2004, Lucas et al. 2007, Sugano and Ishiwatari 2012). Paper-based emanators / devices of metofluthrin were highly effective against *Aedes* and *Ochlerotatus* species with up to 90% reduction in host-landing rate (Lucas et al. 2007, Xue et al. 2012). In experimental rooms, metofluthrin resin-based emanators completely inhibited host-seeking and host-biting behaviors of *Ae. aegypti* laboratory colony, and caused rapid knockdown (KD) and mortality (90% in <1h hr). Using the resin emanators, repellent efficacy lasted for 20 days, which indi-

cates their possible use to replace insecticidal indoor residual spraying (Ritchie and Devine 2013). The repellent activity of the metofluthrin-based OFF! Clip-on device was tested in semi-field conditions at different distances, and the results showed that metofluthrin was highly effective (up to 100% KD and mortality) against *Ae. aegypti* when exposed for 60 min at 0.3 m from the device (Bibbs and Xue 2015). In glass chamber experiments on different mosquito pyrethroid-based coils in Malaysia, metofluthrin was the most effective against *Ae. albopictus* among the compared pyrethroids in regards to KD and killing time (KT). There was considerable variation in mortalities (5-100%) and KT_{50} (2.5-17 min), suggesting the presence of cross-resistance between the tested pyrethroids (Chen et al. 2018). Metofluthrin impregnated nets inconsistently repelled *Ae. aegypti* and *Anopheles dirus* in Thailand based on spatial activity indices measured from sentinel and cone bioassays (Ponlawat et al. 2017). Metofluthrin emanators were moderately effective in reducing the landing rate and KD levels of *Ae. aegypti* (Darbro et al. 2017). Similarly, in Cambodia, slow-release metofluthrin emanators caused variable (47-67%) reduction of landing rates of *Anopheles* species collected in tents or by CDC traps deployed in test premises (Charlwood et al. 2016). Metofluthrin affects host-seeking and biting behavior through a cascade of sequential events of agitation, confusion and knockdown of the target mosquitoes (Buhagiar et al. 2017a). However, prolonged exposure of the mosquito to sub-optimum doses of the active ingredient, especially at the margins of the chemical or device effective range "harbourage area", results in variable responses and the subsequent development of resistance (Buhagiar et al. 2017b). In a cage bioassay mosquitoes exposed to metofluthrin (10% a.i.) for 60 min resulted in reduced mortality in *Ae. aegypti* females, with considerable mortality in males after 40 min. There were no obvious effects on the surviving females' fecundity (Buhagiar et al. 2017b). In Mexico, variable mortalities (41-100%) were reported in *Ae. aegypti* exposed to 13 types of aerosolized insecticides commonly used in houses, due to variable insecticide type, concentration, formulation, and method of application or spraying (Kuri-Morales et al. 2018). In another field study in Mexico, up to 50% reduction in mortality was observed in *Ae. aegypti* susceptible and field resistant strains exposed to aerosol insecticides, plug-in and coil devices, with significant increase in the frequency of KD resistant homozygous allele, *kdr* I1016 (Gray et al. 2018). Widespread pyrethroid resistance in *Ae. aegypti* further confounds effective domestic mosquito management (Estep et al. 2017). Such findings highlight the risks of increased use of house-hold insecticides and repellents for personal protection. New pyrethroid formulations could contain synergists such as piperonyl butoxide or alternatives to enhance insecticide efficacy; or formulations could pair multiple active ingredients, particularly those that mosquitoes do not share resistance between (Bingham et al. 2011, Gray et al. 2018). It is equally important to educate consumers for well-informed use of over-the-counter insecticides and repellent devices to emphasize the role of citizens in integrated management and control programs (Vasques-Prokopec et al. 2017). In an attempt to generate useful information for the aforementioned goals, we report the evaluation of the spatial repellent compound-metofluthrin using the CDC bottle bioassay against susceptible and resistant *Ae. aegypti* strains.

MATERIALS AND METHODS

Female mosquitoes from a pyrethroid susceptible laboratory colony of *Ae. aegypti* 1952 Orlando (ORL) strain were provided by the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology (USDA-CMAVE), Gainesville, FL. This colony was reared at insectary facilities of the Anastasia Mosquito Control District (AMCD) in St. Augustine, Florida. Mosquitoes were maintained at 26 ± 1.0°C, 65–80% relative humidity, and a photoperiod of 14:10 hr (L:D). The adult mosquitoes were provided 10% sugar solution *ad libitum*. For comparison, pyrethroid resistant

Ae. aegypti Puerto Rico (PR) strain was obtained from the USDA-CMAVE. Mosquitoes used were 19 generations of permethrin-resistance and reared under conditions as described in Pridgeon et al. (2008). Briefly, the mosquito eggs were allowed to hatch in a flask and remained overnight until transfer to a plastic tray containing distilled water with larval food. Mosquitoes were reared in an environmentally-controlled chambers set with a temperature profile representing a simulated summer day regimen (ranging from 22 to 30°C) and 80% RH. Incandescent lighting was set to a crepuscular profile with a photoperiod of 14 hr:10 hr (L:D), including 2 hr of simulated dawn and 2 hr of simulated dusk. Adults were held in a screened cage and provided 10% sucrose *ad libitum*. All mosquitoes selected for testing were 5-8 d old, non-blood-fed females. Technical grade 98.1% permethrin (45614 Pestanal, Sigma-Aldrich Co. LLC, St. Louis, MO) and 95.6% metofluthrin (SumiOne, Sumitomo Chemical Company Ltd., Tokyo, Japan) insecticides were dissolved in 1 ml of acetone to treat 250 ml glass bottles according to procedures outlined in Brogdon and McAllister (1998). The final concentration was 43 ng/ bottle for each insecticide. Each bottle was filled with 15-20 *Ae. aegypti* adults and then observed for a maximum of 120 min. For each replicate, three bottles were assigned to ORL strain, three bottles were assigned to PR strain, and one bottle each was assigned to a control per insecticide. A total of 16 bottles were used each time. Permethrin and metofluthrin were replicated three times on different dates. The mean number of dead/moribund mosquitoes in bottles was plotted over time for each insecticide and the control. The time-mortality relationship lines were plotted and the median lethal KT values were deduced from the lines for each strain and each insecticide tested. For comparison of efficacies and susceptibility level of each strain, the resistance ratios (RR) were calculated by dividing the KT-50 values of the resistant strain by the susceptible strain. Abbott formula was not performed due to almost zero number of mortality in control group.

RESULTS AND DISCUSSION

Insecticide susceptibility and resistance of both permethrin and metofluthrin were detected in the PR strain as compared to the ORL strain (Fig. 1 and Table 1). The RR (KT_{50}-PR/KT_{50}-ORL) was 30 and 15 folds for permethrin and metofluthrin, respectively.

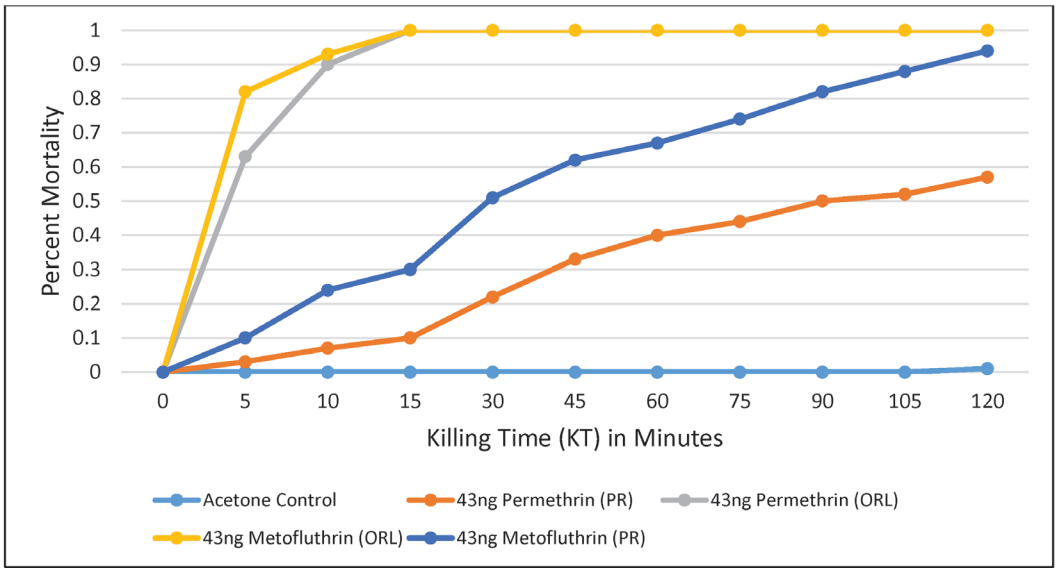

Figure 1. The time-mortality relationships for Orlando susceptible lab (ORL) and Puerto Rico resistant lab (PR) strains of *Aedes aegypti* exposed to permethrin and metofluthrin using the CDC bottle insecticide bioassay.

Table 1. Lethal Killing Time (KT) in minutes and resistance ratio (RR) values for Orlando susceptible lab (ORL) and Puerto Rico resistant lab (PR) strains of *Aedes aegypti*.

	Killing time (KT) in minutes						Resistance ratio (RR)			
	Aedes aegypti PR Strain			*Aedes aegypti* ORL Strain			PR Strain		ORL Strain	
	KT_{50}	KT_{90}	KT_{100}	KT_{50}	KT_{90}	KT_{100}	KT_{50}-PR/KT_{50}-ORL	KT_{50}-Per/KT_{50}-Met	KT_{50}-Per/KT_{50}-Met	Notes
Permethrin (43ng)	90	NA	NA	3	10	15	30	3	1.5	PR: KT 57% at 120 min
Metofluthrin (43ng)	30	110	NA	2	8	15	15	1	1	PR: KT 94% at 120 min

The PR strain was less resistant to metofluthrin than to permethrin, with RR (KT_{50}-PR-per/KT_{50}-PR-met) of three-fold. However, it is clear that substantial cross resistance is present in the permethrin-resistant mosquitoes. Exposure of the PR strain to permethrin for 120 min caused only 57% mortality, while metofluthrin caused 94%. The susceptible ORL strain had comparable susceptibility to both permethrin and metofluthrin, where KT_{100} was <15 min. In this report, the permethrin-resistant *Ae. aegypti* PR strain showed moderate level of cross-resistance to metofluthrin, with RR of 30- fold and 15-fold for permethrin and metofluthrin, respectively. This result is similar with results from lab and field experiments reported by Chen et al. (2018), Kuri-Morales et al. (2018), and Gray et al. (2018). Observed reductions in susceptibility are due to the long-term and inconsistent exposure of the mosquitoes to sub-lethal doses of the insecticide active ingredients used in different formulations, whether through space spraying, residual spraying, or vapor repellents (Kuri-Morales et al. 2018, Gray et al. 2018). In the State of Florida, there was wide-spread *kdr* resistance in *Ae. aegypti* field strains (RR of 6-61 fold to permethrin) with significant increase in the frequencies of homozygous and heterozygous *kdr* alleles of V1016I and F1534C (Estep et al. 2018). In contrast, in the same study, lower levels of resistance were reported in *Ae. albopictus* strains, even though they were collected from the same geographies that contained resistant *Ae. aegypti* strains. These various studies show the complex nature of *kdr* resistant dynamics and inheritance in different mosquito species or strains (*Ae. aegypti* and *Ae. albopictus*) and the potential spread of cross-resistance between different pyrethroids commonly used for vector control. Therefore, for better monitoring and management of resistance at early stages, it is essential to measure the susceptibility levels of various mosquito populations using a combination of bioassays (WHO, CDC bottle, and topical application bioassays) and molecular assays (genotyping and allele frequency) (Reid et al. 2014, Al-Nazawi et al. 2017, Estep et al. 2017, 2018). Chemical

adulticides are still the main means of controlling mosquito vectors of diseases. Whether by aerosol or repellents, pyrethroids are the most used insecticide as spatial insect repellent compounds for personal protection against the bites of adult mosquitoes in domestic and area-wide scenarios. Spatial repellent volatile pyrethroids are highly insecticidal, and their use to control mosquitoes is increasing. The combined pressures from pyrethroids deployed in home use, commercial agriculture, and vector control has resulted in development of *kdr* in response to almost all pyrethroid active ingredients (Wagman et al. 2015, Estep et al. 2018, Gray et al. 2018). Sustainable mosquito control programs hinge on routine monitoring of insecticide efficacy. This can be performed through both conventional insecticide susceptibility bioassays (the WHO and CDC bioassays) and molecular genotyping of resistance genes such as *kdr* alleles (Estep et al. 2018). Our findings show that the permethrin-resistant PR strain is highly resistance to both permethrin and metofluthrin compared to the susceptible ORL strain, and the PR strain is less resistance to metofluthrin than to permethrin.

ACKNOWLEDGEMENTS

We would like to thank McLoughlin Gormley King (MGK) Company and Sumitomo Chemical Company for providing technical grade metofluthrin and partial fund and USDA- CMAVE/Mosquito and Fly Unit (JJ Becnel's laboratory), Gainesville, FL for providing the resistant strain of *Ae. aegypti* for this study. The Anastasia Mosquito Control District does not endorse specific commercial products. Mention of propriety goods are for research purposes only.

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SCIENTIFIC AND OPERATION NOTES

FIELD COMPARISON OF AUTOCIDAL GRAVID OVITRAPS AND IN2CARE TRAPS AGAINST *AEDES AEGYPTI* IN DOWNTOWN SAINT AUGUSTINE, NORTHEASTERN FLORIDA

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ABSTRACT

Mosquito Control programs are utilizing cost-effective long term autocidal gravid traps because they minimize labor needs while targeting the gravid population of container-breeding mosquitoes. This field study compared the efficacy of the In2Care Mosquito Trap and the Centers for Disease Control and Prevention autocidal gravid ovitrap (CDC-AGO). The study consisted of two control and two treatment sites, and each treatment site had either 100 In-2Care Mosquito Traps or 100 CDC-AGOs. *Aedes aegypti* populations in each site were monitored using Biogent (BG) Sentinel 2 mosquito traps and ovitraps. Analysis of pre- and post-treatment data indicated no significant difference in adult mosquito populations detected by BG traps from either the In2Care or CDC-AGO sites. However, the mean number of eggs collected by ovitraps showed significant reduction in both trap type treated areas posttreatment, compared to pre-treatment. Furthermore, the mean number of egg collections from the In2Care mosquito trap treated area was much less than the collection from the CDC-AGO trap treated area post-treatment.

Key Words: *Aedes aegypti*, Autocidal Gravid Ovitraps, In2Care mosquito trap, Gravid Mosquitoes

Florida mosquito control districts focused on the control of *Aedes aegypti* Linn. during the outbreak of Zika virus in South America and Florida in 2016 (Smith et al. 2018). At the same time, the Anastasia Mosquito Control District (AMCD) noted an increase in *Ae. aegypti* populations primarily in historic downtown Saint Augustine (Dixon et al. 2020), a high traffic tourist area and one of the pillars of Saint Augustine's economy. The control of *Ae. aegypti* populations were targeted using a door-to-door treatment approach with cultural and chemical, larval and adult control practices which included source reduction, larviciding permanent water sources, adulticide treatment with handheld foggers, and community education. Despite all those efforts, *Ae. aegypti* populations continued to persist (Xue et al. 2020). Considering conventional treatment efforts failed to have an impact on mosquito populations in downtown Saint Augustine (Xue et al. 2020), new mosquito abatement tactics targeted at container-breeding *Aedes* mosquitoes were needed. Some novel strategies for the control of *Aedes* mosquitoes include Sterile Insect Technique (SIT), Incompatible Insect Technique (IIT), transgenic technologies, In2Care traps, and Center for Disease Control and Prevention's Autocidal gravid ovitraps (CDC-AGOs).

CDC-AGOs are dual action control and surveillance tools aimed at capturing and killing gravid female container-breeding mosquitoes. The CDC-AGOs are comprised of a container and infusion water to simulate suitable larval habitats. Contained within CDC-AGOs are either chemical or non-toxic

mosquitocidal agents used to induce mortality immediately or a few days after contact. The novel CDC-AGOs have been previously tested for the control of container-breeding *Aedes* mosquitoes in Puerto Rico where they reduced *Ae. aegypti* populations by 60-80% when deployed with an 85% coverage in the treatment area (Barrera et al. 2014a, 2014b). The lower vector densities from CDC-AGO trapping in the treatment area also reduced the transmission of Chikungunya virus (Barrera et al. 2016).

Unlike the CDC-AGO traps that mainly target gravid adult females, In2Care mosquito traps potentially target multiple life stages of the mosquito (Buckner et al. 2017, Su et al. 2020). In fact, evidence from a study using the In2Care mosquito traps indicated all stages of the mosquito life cycle were targeted from the combined use of *Beauveria bassiana* and pyriproxyfen (Snetselaar et al 2014). Pyriproxyfen and *Beauveria bassiana* contaminate *Aedes* mosquitoes after exposure to the water and inner surface of the trap. As *Aedes* mosquitoes engage in skip oviposition behavior, the mosquitoes contaminated with pyriproxyfen can contaminate multiple container habitats and affect the development of larvae and pupae. Adults suffer increased mortality after three days of exposure to *Beauveria bassiana*, yet live long enough to contaminate multiple containers with pyriproxyfen. Clearly, CDC-AGO traps and In2Care mosquito traps were shown to be effective when tested previously (Buckner et al. 2017, Cilek et al. 2017, Su et al. 2020). However, a direct comparison of CDC-AGO and In2Care mosquito traps to determine the most effective trap against *Ae. aegypti* in the field has not been assessed. In this study, AMCD compared both CDC-AGO and In-2Care mosquito traps in two sites within the downtown area of Saint Augustine, Florida. This study should help mosquito abatement districts find alternative strategies to control the populations of container-breeding mosquitoes in metropolitan areas.

Two sites were chosen in the downtown Saint Augustine, Florida based on their high abundance of *Ae. aegypti*. The treatment sites were 7.3 hectares in size and 700 meters apart. Each treatment site also had its own control site which wrapped around the treatment area no more than 300 meters. The CDC-AGO site had 91 homes and the In2Care site had 84 homes. Each treatment site had either 100 CDC-AGOs or 100 In-2Care mosquito traps as test traps.

The CDC-AGO trap provided by Spring-Star is a black 19 L bucket with a fitted lid that houses a removable capture chamber. The capture chamber encloses a fitted sticky board and a small mesh screen on the bottom side of the capture chamber which ensures the mosquitoes do not have access to the water. The CDC- AGO trap requires 8 liters of water and a small bundle of hay; no pheromones or pesticides are required. Machined slots at the 8-liter mark prevent excess filling from rain or irrigation. The CDC-AGO traps were placed in discrete locations at 1-2 traps per home.

The In2Care mosquito traps (provided by UNIVAR) is a small black bucket trap shaped like a planter pot. The trap lid has a 2.5 cm gap to the bucket's rim that allows for mosquito entry but excludes debris and animals from the water inside. Slots on the top of the trap drain excess water in the event of rain storms and irrigation. This trap requires 3.5 liters of clean tap water and provided with pesticide-treated gauze (Pyriproxyfen, *Beauveria bassiana*, and Silicon Dioxide) which are placed onto a floating ring to keep the gauze upright. Two tablet attractants from the original trap set are added to the water to attract container-breeding mosquitoes. The In2Care mosquito traps were also placed in discrete locations at about 1-2 traps per home.

All 200 traps, 100 of each type per treatment area, were set by a mosquito control technician and summer intern during a mosquito outbreak following Hurricane Irma over a two-day period. In2Care mosquito traps were set from September 18th to September 19th then CDC-AGO traps were set from September 21st to September 22nd.

Pre- and post-treatment surveillance was conducted by using 24 oviposition cups (ovitraps) and 12 Biogents Sentinel 2 Traps (BG traps). Pre-surveillance was done two weeks

before the test traps were placed in the treatment area, and populations were monitored weekly for two months after trap placement in the field.

Three BG traps were placed throughout each treatment and control site (6 BG traps for the CDC- AGO area, 6 BG traps for the In2Care trap area). Traps were operated for 24 hours weekly and each collection was returned to the lab and evaluated for the number of *Ae. aegypti*, *Ae. albopictus*, and other species collected in the traps.

Six ovitraps were placed in each treatment and control site to monitor egg production from gravid container breeding mosquitoes. The ovitraps were black and could hold up to 473 mL of water. A stock solution of infusion water was made from 24 grams of orchard hay and 3 liters of water and fermented for seven days. Each trap was fitted with seed germination paper and filled with 237 mL of stock infusion water diluted by 10%. To avoid overfill, a small hole was drilled above the 240 mL mark. Every week, the seed germination paper and infusion water were replaced. After weekly collections, the eggs were counted under a microscope.

The weather throughout the testing period was consistent, with the temperature gradually getting cooler as the evaluation continued into November. Precipitation was also consistent in September and October, but there was an increase in precipitation during November. Overall, residents were receptive to the traps being placed at their property, and some requested that they keep the traps after the testing period.

All statistical analyses were done using JMP statistical software. We explored the effects of CDC-AGO and In2Care mosquito traps on adult *Ae. aegypti* abundance and egg oviposition rates using a Shapiro-Wilk goodness-of-fit test along with a Kruskal-Wallis test. Our significance levels were set to 0.05. Also, Mulla's formula was used to estimate the percent reduction of the adult mosquito population in the treatment areas (Mulla et al. 1971).

Table 1 shows the mean numbers (%) of adult *Ae. aegypti* collected by BG traps baited with BG Lure and CO2 during pre- and post-treatments. Kruskal-Wallis analyses indicated no significant reduction in adult *Ae. aegypti* abundance post-treatment, compared to pre-treatment (P= 0.113, df = 3, F= 2.0624). In addition, an analysis using Mulla's formula suggested a dramatic increase in adult *Ae. aegypti* population post-treatment (52% in the In2Care treatment area, 104% increase in the CDC-AGO treatment area). There was a two-week gap between the pre-treatment and post-treatment periods due to Hurricane Irma and a mosquito outbreak. The trapping was also not conducted on the weeks of October 5th and October 19th due to another mosquito outbreak.

There are multiple reasons that could explain the ineffectiveness of both sets of traps for adult population of mosquitoes: trap malfunction, weather anomalies, and reinvasion of *Ae. aegypti* from surrounding areas. First, trap malfunction, especially with the In2Care mosquito traps, was observed during the study. Out of the 100 In2Care mosquito traps that were deployed in the treatment area, 20% were dry but sitting upright, 20% were knocked over, and 8% were missing. The In2Care mosquito traps seemed to easily fall over due to instability and a top-heavy structure. The top of the traps extended above the base which required multiple pieces to make a complete shaft to hold it in place. When the top was hit, the water and the top itself would easily shift

Table 1. Mean (% ± Standard Error) adult mosquitoes caught per night in Biogents Sentinel 2 traps in control and treatment areas both before (Pre-treatment) and after (Post-treatment) test trap deployment.

	Pre-treatment	Post-treatment
CDC-AGO - Control	13.83 (± 4.53)	7.66 (± 1.63)
CDC-AGO - Treatment	10.83 (± 2.5)	15.80 (± 3.68)
In2Care - Control	21.70 (± 7.04)	8.00 (± 2.06)
In2Care - Treatment	9.30 (± 3.20)	7.60 (± 1.49)

Table 2. Mean (± Standard Error) eggs oviposited per week in ovitraps of control and treatment areas both before (Pre-treatment) and after (Post-treatment) test trap deployment.

	Pre-treatment	Post-treatment
CDC -AGO- Control	9.08 (± 3.89)	2.74 (± 1.62)
CDC- AGO - Treatment	29.83 (± 9.38)	10.50 (± 3.46)
In2Care - Control	13.83 (± 7.68)	0.26 (± 0.16)
In2Care - Treatment	19.08 (± 7.51)	1.17 (± 0.57)

resulting in the top coming apart from the trap. Also, trap failures could have occurred due to home owner dumping the infusion water and strong wind gusts toppling them. For CDC-AGO traps, upon deployment the top of the trap was unstable and would fall off. The CDC-AGO trap locations experienced similar conditions as the In2Care sites. Despite the tops coming off upon deployment, the CDC-AGO traps design was sturdy enough that only 10% of the traps were damaged or missing. The damaged and missing traps may have been due to people removing traps, debris clogging or disabling the screen on the top of the trap, or severe weather (described below) toppling or damaging the capture chamber. Since the writing of this publication, both the In2Care and CDC-AGO traps have undergone modifications to improve the stability and hardiness of the traps in harsh weather conditions.

During this study, Hurricane Irma caused heavy flooding, strong winds, abnormally high tides, and the destruction of environmental and artificial structures (roofs, trees, telephone poles, lawn décor, etc.) in both treatment and control sites one week prior to trap deployment. The intense wind and rain left debris in hard to reach areas as well as stacks of debris awaiting removal by Saint Johns County Public Works for an extended period of time. This excess debris may have created new breeding sites for *Ae. aegypti* which could have led to reinvasion into the treatment areas. Also, the intense wind and rain that came from multiple storms possibly flushed out the pyriproxyfen tainted containers in the In2Care mosquito traps resulting in pre-treatment like conditions.

Table 2 shows the mean numbers of *Aedes* eggs collected by ovitraps during pre- and post-treatment surveillance. The mean numbers of eggs collected by ovitraps were reduced at 35% in the CDC-AGO trap treated area and at 61% in the In2Care mosquito trap treated area post treatment, compared to pre-treatment ($P = 6.334$, df = 3, $P < 0.01$). The mean number of eggs collected by ovitraps from the In2Care mosquito trap treated area was approximately 80% lower than the number of eggs collected from the CDC-AGO trap treated area in the post treatment.

In summation, this study directly compared the effectiveness of CDC-AGO and In2Care mosquito traps. However, both trap types did not show significant reduction of the adult population of *Ae. aegypti*, but reduced the mean number of eggs oviposited in the treatment areas post-treatment, compared to the pre-treatment. Likely factors that contributed to failure for reduction of adult mosquito population include trap malfunctions, excessive larval sources from hurricane Irma, and mosquito re-invasion. Additional investigations of mass-trapping and population monitoring schemes are needed to enhance their effectiveness in the field.

ACKNOWLEDGMENTS

We would like to thank SpringStar and UNIVAR for providing traps for the study, M. Smith, M. Duet for technical help, and James Cilek for his advice on experimental design and statistical analyses.

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BARRIER TREATMENTS USING COMBINED LAMBDA-CYHALOTHRIN AND PYRIPROXYFEN REDUCE PERIDOMESTIC *AEDES* MOSQUITOES IN A SUBTROPICAL ENVIRONMENT

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Subject Editor: Seth Britch

ABSTRACT

Barrier treatment of vegetation using lambda-cyhalothrin has been shown to be effective at reducing adult mosquito populations in the US. However, recent investigations have indicated that standard residual adulticide barrier treatments may be enhanced when combined with an insect growth regulator targeting immature stages that could be transferred to immature habitat by adults contacting treated surfaces. We conducted field trials at residential sites in a subtropical urban environment in north central Florida treating blocks of vegetation with residual sprays of lambda-cyhalothrin (Demand® CS) and pyriproxyfen (Archer®) alone and in combination treatments to determine their efficacy against peridomestic mosquitoes. The combined treatment resulted in consistent approximately 100% reduction in *Aedes* mosquito eggs for 16 wk post-treatment compared to not significantly lower but more variable reductions at alone treatment sites.

Key Words: barrier treatment; disease vectors; insect-growth regulator; pyrethroid; residual adulticide

Peridomestic barrier treatments to surrounding vegetation for control of adult disease vector mosquitoes have shown efficacy of 85–100% reduction in collection of adult mosquitoes in urban settings and could be considered a proven method for urban mosquito control (Trout et al. 2007, Unlu et al. 2018, Stoops et al. 2019). Recently, however, studies have been conducted in New Jersey in the northeastern US to enhance this control technique by combining an insect growth regulator with the residual adulticide that could be transferred to immature habitat by females that contacted the treated barrier and survived long enough to oviposit (Unlu et al. 2018, Williams et al. 2019). In this study we investigated whether this enhanced barrier treatment technique could be effective in a warm subtropical habitat in the southeastern US in north central Florida.

We conducted field applications to evaluate the residual efficacy of lambda-cyhalothrin (Demand® CS; Syngenta Crop Protection, Inc., Greensboro, NC) and pyriproxyfen (Archer®; Syngenta Crop Protection, Inc., Greensboro, NC) against peridomestic mosquitoes as a barrier treatment in nine approximately 1 A residential sites with dense foliage perimeters on the University of Florida campus in Gainesville, FL. Three sites were randomly selected for treatment with 0.06% lambda-cyhalothrin alone, three

for combined treatment with 0.06% lambda-cyhalothrin + 0.01% pyriproxyfen, and three for untreated controls. Limited resources precluded investigation of 0.01% pyriproxyfen on its own. We used a Stihl® SR 200 (STIHL Inc., Virginia Beach, VA) backpack mist blower on the number three setting for all barrier spray treatments, with a target application rate of 0.61 L/min. All field trials were conducted during July 5–October 18, 2016.

We measured efficacy of the barrier treatments by deploying five 450 mL ovicups (Creative Converting Inc., Clintonville, WI) filled with 150 mL of tap water and a 15 cm x 2 cm wooden tongue depressor (Fisherbrand, Pittsburgh, PA) during pre- and post-treatment periods in each study site to assess *Aedes* mosquito egg density. The tongue depressors were collected and replaced every 7 d and returned to the laboratory for egg counts. The ovicups for the pre-treatment period were deployed two weeks before spray applications to establish the baseline density of *Aedes* eggs expected in the area from the natural populations. We applied two rounds of barrier treatment applications on the vegetation bordering each of the 6 treated sites: the first applied two weeks after collection of pre-treatment data, and the second applied eight weeks after the first treatment.

Efficacies of treatments were evaluated by egg counts at twelve intervals consisting of 1-8, 10, 12, 14, and 16 wk after the initial application. We downloaded publicly available weather data throughout field trials from the weather recorder at the Gainesville, FL regional airport approximately 8 km from study area. Mean egg densities were calculated for all sites for each sample period to determine the variance in egg density within each treatment – i.e., lambda-cyhalothrin, lambda-cyhalothrin + pyriproxyfen, and untreated control – over time and evaluate differences using a three-way *t*-test at the $P < 0.05$ level using JMP version 12 (Boblingen, Germany).

The distributions of egg collection data for all sites over the 2 wk pre-treatment period are shown in Fig. 1, and the three-way *t*-test confirmed there were no significant differences among the mean number of *Aedes* mosquito eggs across the 9 sites designated for alone and combined treatments and untreated control (Table 1). Regarding post-

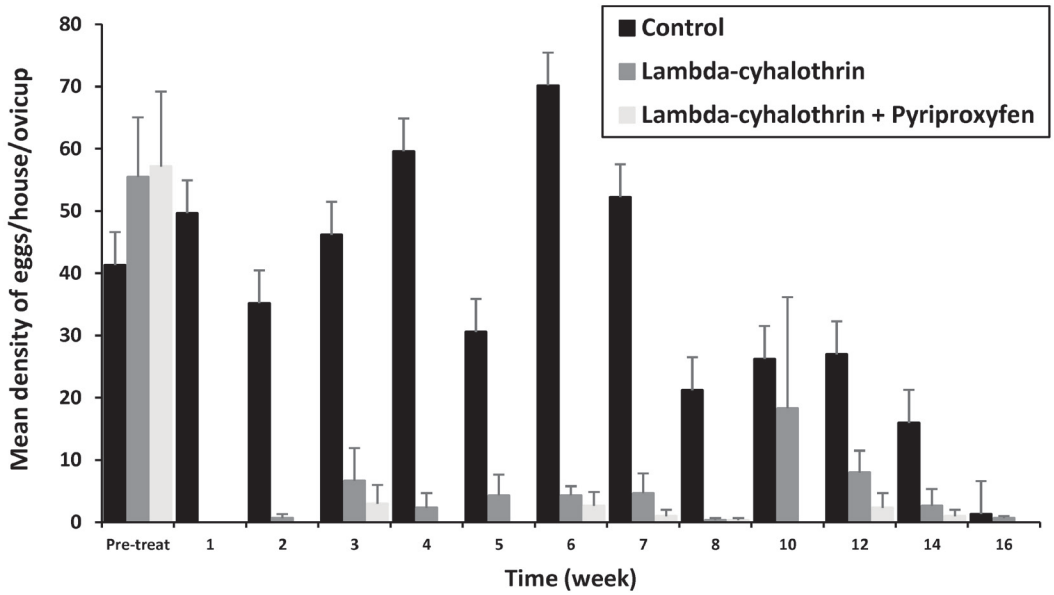

Figure 1. Mean number (standard error bars) of *Aedes* eggs collected by ovitraps per site (5 per site) for 2 wk pre-treatment and for 16 wk following alone and combination residual barrier treatments. The alone treatment consisted of 0.06% lambda-cyhalothrin, and the combined treatment consisted of 0.06% lambda-cyhalothrin + 0.01% pyriproxyfen.

Table 1. The number of *Aedes* mosquito eggs (mean ± SE) collected from 9 study sites in Gainesville, FL for 2 wk before and 16 wk after application of alone and combined barrier treatments.

Treatment (3 sites each)	Pre-treatment Mean ± SE	Post-treatment Mean ± SE
lambda-cyhalothrin 0.06%	55.5 ± 9.57[a]	4.3 ± 1.59[a]
lambda-cyhalothrin 0.06% + pyriproxyfen 0.01%	57.2 ± 12.02[a]	0.9 ± 0.30[a]
Untreated Control	41.3 ± 31.8[a]	38.6 ± 7.61[b]

Means followed by the same letter in the same column are not significantly different ($P > 0.05$).

treatment egg collection data, there was an approximately 100% reduction in oviposition by *Aedes* mosquitoes at sites treated with the combined lambda-cyhalothrin and pyriproxyfen treatment for up to 16 wk (Fig. 2). For the alone treatment with lambda-cyhalothrin, we observed a reduced (but non-significant) efficacy compared to the combined treatment sites with a mean value of 4.3 *Aedes* eggs post-treatment (Table 1). This non-significant overall reduction in efficacy shown in Table 1 between the combined and alone treatments is likely due to the reduced percent reductions compared to controls shown by the alone treatment in collections from weeks 10, 12, and 16 in Fig. 2. One explanation for this is that significantly elevated rainfall of 202 mm took place in the study area in September 2016, corresponding to egg collections at weeks 10 and 12, compared to monthly rainfall of 38–83 mm for July–August. Similarly this heavy rain event appeared to slightly decrease the impact of the combined residual barrier treatment at the 12 wk and 14 wk collections when compared side by side with controls (Fig. 1) and as a percent reduction compared to controls for the same collection speriods in Fig. 2.

Our results indicate that the combination of lambda-cyhalothrin and pyriproxyfen in a peridomestic residual barrier treatment enhanced the treatment in two ways compared to lambda-cyhalothrin on its own. First, the combined treatment resulted in consistently,

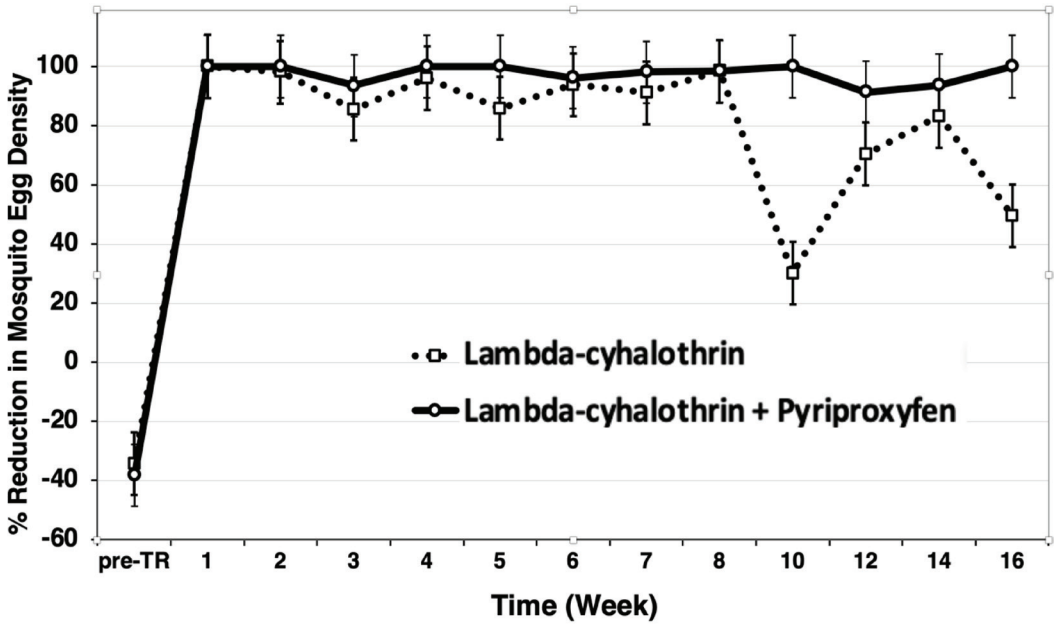

Figure 2. Percent reduction over 2 wk pre-treatment and 16 wk post-treatment in mean *Aedes* mosquito egg density collected at alone 0.06% lambda-cyhalothrin and combined 0.06% lambda-cyhalothrin + 0.01% pyriproxyfen residual barrier treatment sites compared to untreated control sites.

though not significantly, greater reduction in egg collections throughout the study period. Second, the combined treatment provided more consistent performance through elevated rainfall conditions compared to the traditional barrier treatment on its own.

We hypothesize that in our investigation the combined treatment provided an integrated impact on adult and immature life stages that caused greater (though not significantly) and more consistent reductions in mosquito populations compared to alone treatments. Lambda-cyhalothrin is a synthetic pyrethroid adulticide and causes rapid knockdown effect to susceptible adult population of mosquitoes; pyriproxyfen indirectly reduces the number of adult mosquitos by slowing down the development of mosquito immatures and lead to failure in adult emergence. The combination of the larvicidal and adulticidal products theoretically increased the efficacy of the application by preventing the development of immature stages and survival of adult mosquitoes within the treated areas, and limiting survival of mosquitoes flying in from nearby untreated areas.

Future studies should include sites treated with a residual barrier treatment of pyriproxyfen on its own to tease apart the relative contributions of each active ingredient in the combined treatments. In addition, testing of the water in ovicups could detect whether pyriproxyfen is present, which could help determine the relative contributions of the adulticide and the insect growth regulator to reductions in egg collections. Future studies should also simultaneously collect adult *Aedes* mosquitoes in addition to egg collections across the study area to better evaluate whether residents could expect enhanced reductions in biting pressure from combine treatments. Finally, further investigation should be focused on whether the combined treatments can create a more weather-resistant integrated system that provides stable long term control.

ACKNOWLEDGMENT

This is a research report only and the mention of commercial products is to provide specific information and does not imply endorsement by University of Florida, New Orleans Mosquito and Termite Control Board, or University of Nevada-Reno. The research reported in this publication was supported by Syngenta; however, the content is solely the responsibility of the authors and does not necessarily represent the official views of Syngenta.

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EVALUATION OF ORANGE OIL APPLIED BY THREE BACKPACK SPRAYERS AGAINST *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

A solvent orange oil has been used to mix with permethrin and PBO as a commercial adulticide product called Aqualure® 20-20 for control of adult mosquitoes. The orange oil at 2.7% and 3.5% sprayed by three backpack sprayers, Hudson battery operated sprayer modified with a Solo nozzle, hand pump sprayer Solo-425, and Birchmeire battery operated sprayer, against caged adult female *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say resulted in 89%–100% mortality of *Ae. aegypti* and 100% mortality of *Cx. quinquefasciatus*. The three different backpack sprayers did not show any significant differences in the percent mortality. Our test results demonstrate that orange oil alone at a high dose (3.5 %) showed effective insecticidal characteristics against both species of adult mosquitoes.

Key Words: permethrin, orange oil, adulticide, *Aedes aegypti*, *Culex quinquefasciatus*

Orange oil is an essential oil extracted from the peel of the orange fruit. The essential oils extracted from the orange plant have shown insecticidal properties against mosquitoes and other insects (Ezeonu et al. 2001, Norris et al. 2015, Badawy et al. 2018). Orange oil varies in chemical composition depending on the fruit and method of extract action. The oil not only proves to be an effective repellent, but it has also shown to be lethal to adult mosquitoes (Xue et al. 2003, Phasomkusolsil and Soonwera 2011, Badawy et al. 2018) and can act as a synergist for pyrethroid insecticides (Gross et al. 2017). Orange oil has been used to mix with permethrin and piperonyl butoxide (PBO) as Aqualure® 20-20 (All Pro Vector Group, Bloomington, MN) adulticide and marketed for adult mosquito control (Amoo et al. 2012). Recently, two new backpack sprayers operated with batteries are available commercially. The objectives of this study were to determine the orange oil's insecticidal efficacy against two species of adult mosquitoes and to evaluate the two new battery-operated backpack sprayers, compared with the standard hand-pump sprayer Solo 425® (Solo Inc., Newport News, VA) against caged adult mosquitoes.

The colonies of the Orlando strain of *Ae. aegypti* Linn. and the Gainesville strain of *Cx. quinquefasciatus* were provided by the USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL and reared at Anastasia Mosquito Control District (AMCD), St. Augustine, FL. Adult female mosquitoes at 5-7 days old were used for the testing.

The three backpack sprayers, Hudson battery-operated sprayer (13854 Never-Pump Back-Pak®, H.D. Hudson Manufacturing Co., Chicago, IL) which was modified by using an extra sprayer nozzle from Solo, hand pump sprayer Solo 425, and Birchmeier battery-operated sprayer (REC 15, Birchmeier Spruhtechnik AG, Stetten, Switzerland), described by Conover et al (2015), were calibrated and used for the testing. Distilled water was sprayed from each sprayer into a measurable plastic container for 1 min to quantify spray volume. The Solo sprayer was pumped every 3 sec to maintain constant pressure while calibrating. Modified Hudson sprayer's flow rate was 728 mL/min, Solo was 693 mL/min,

and Birchmeier was 735 mL/min. Orange oil was provided by All Pro Vector Group (Bloomington, MN). The tested solution was diluted by distilled water at the low rate 2.7% and high rate 3.5%. All three sprayers produced a spray distance of approximately 2 meters. The certified applicator's walking speed was 6.4 km/h.

The study site was located in the back yard of AMCD facilities located at East Pope Road, St. Augustine, FL (29.859515, 81.279366). For each test, 12 mesh-screened (0.7mm mesh size) cylindrical paper cages (9 for treatments and 3 for controls) containing 10 female mosquitoes at 5-7 days old per cage transferred via mouth aspiration were used. The same number of mosquitoes and type of cages were used for each application (a total of 36 cages for 3 sprayers). The cages were adhered to vertical PVC pipes (1.5 meters) within the spray path. The 9 pipes were placed 3 meters apart. The control cage mosquitoes were placed at an appropriate distance (approximately 17 meters) outside of the spray path to prevent insecticide drift from the treatment applications. The treatment cages were sprayed from approximately 2 meters away in a waving motion while the applicator was walking at 6.4 Km/h. The caged mosquitoes sat for 15 min after exposure then a knockdown count was taken. Post-exposure cages were brought back to the AMCD laboratory and maintained on a 10% sucrose solution. Mortality of mosquitoes was read at 24 hours after exposure. The same experiments were repeated three times. Weather conditions averaged 27° C temperature, 70% relative humidity, and 4 Km/h wind speed.

The Fig. 1 showed that orange oil at the high dose (3.5%) resulted in 100% mortality of *Cx. quinquefasciatus* at 24 h, sprayed by all three types of backpack sprayers. The oil at the low dose (2.7%) sprayed by Solo, Hudson, and Birchmeire sprayers against *Cx. quinquefasciatus* resulted in 97%, 91%, and 84% mortality at 24 h, respectively. The oil at the high dose sprayed by Solo, Hudson, and Bichmeire against *Ae. aegypti* resulted in 82%, 97%, and 100% mortality at 24 h, respectively. The oil at the low dose (2.7%) sprayed by Solo, Hudson, and Birchmeire sprayers against *Ae. aegypti* resulted in 47%, 76%, and 71% mortality at 24 h, respectively. It is not surprising that the high dose of orange oil at 3.5% caused significantly higher mortality (F=4.366, $P < 0.01$) of both species of mosquitoes, regardless of the types of backpack sprayer, compared with the mortality caused by the low dose at 2.7%. The three types of backpack sprayers did not show any significant difference ($P > 0.05$) against either species of caged mosquitoes although Solo sprayer caused lower mortality against the two species of adult mosquitoes at the high and low dose of the oils.

Norris et al (2015) reported that commercial plant essential oils possess insecticidal characteristics against adult mosquitoes. Our results support Norris et al's findings and confirm the insecticidal activity of orange oils against adult mosquitoes. Other benefit from the commercial plant essential oils is the capability of increasing the efficacy of permethrin against *Ae. aegypti* and *Anopheles gambiae* (Gross et al 2017). These factors explain why orange oils have been used for the commercial product formulation of the permethrin-based Aqualuer® 20-20 for the control of adult mosquitoes.

Back pack sprayers are convenient means for application of liquid insecticide products (Kardatzke et al. 1981, Xue et al. 2012). Conover et al (2015) reported that the three same backpack sprayers sprayed Aqualuer® 20-20 against caged *Ae. aegypti* resulted in no significant difference in percent mortality of the test mosquitoes between the sprayers, but the Birchmeier sprayer was the preferable machine in terms of its physical characteristics and operator use with battery. The orange oils sprayed by the three backpack sprayers against caged *Ae. aegypti* resulted in similar mortality and are in agreement with Conover et al (2015). Thus based on our results, either one of the three backpack sprayers could be used for adult mosquito control based on the availability and cost, but the battery-operated backpack sprayer may be more convenience.

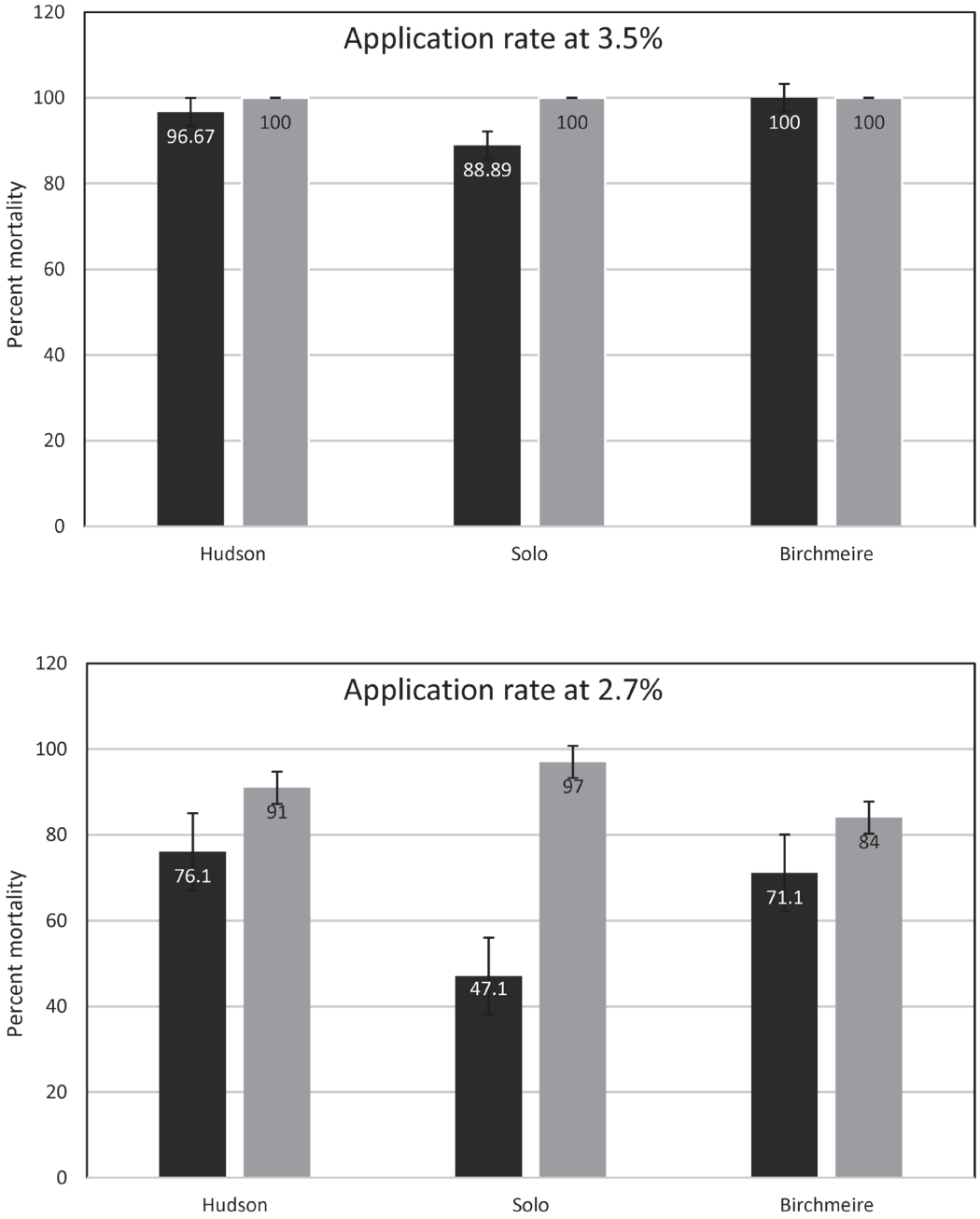

Figure 1. Mortalities (mean % ± SE) of caged adult female *Aedes aegypti* (bold black) and *Culex quinquefasciatus* (light black) at 24 h after exposed to orange oils (high rate at 3.5% & low rate 2.7%) sprayed by three different backpack sprayers.

ACKNOWLEDGMENTS

Thanks All Pro LLC for providing the solvent orange oils for the testing, D. Conover, M. Smith, and K. Gaines for their participations in the experiment and technical help. This is a research report only and does not mean AMCD to endorse any commercial products.

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EVALUATION OF D-ALLETHRIN IN THE THERMACELL MOSQUITO REPELLENT DEVICE AGAINST THE LONE STAR TICK UNDER LABORATORY CONDITIONS

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ABSTRACT

D-allethrin vapor generated from a personal mosquito repellent device (Thermacell MR300) was evaluated for its effectiveness to repel the lone star tick, *Amblyomma americanum* (adults and nymphs) when released at tick body level in a wind tunnel and in an olfactometer. In the wind tunnel 48.5% of ticks moved upwind when only attractant lure was present, while only 30.8% moved upwind when d-allethrin repellent was present with the lure. In the olfactometer strong repellency of d-allethrin vapor to adults was observed, but the effect was reduced with nymphs. Results of this study showed that d-allethrin vapor generated by the Thermacell MR300 pad could be used to reduce movement of ticks towards a host under some conditions.

Key Words: *Amblyomma americanum*, attractant, olfactometer, repellent, ticks, wind tunnel

Humans are at high risk of tickborne diseases but are not adequately protected from these arthropod threats (De la Fuente and Estrada-Pena 2012, Madison-Antenucci et al. 2020). In the United States, reported tickborne disease cases more than doubled from 2004 to 2018 to an all-time high and Lyme disease accounted for more than 70% of all cases in 2017 (CDC 2020). These demonstrated patterns of increased risk of tick-borne disease to humans call for improved technologies for surveillance, pathogen detection, prevention, and control of ticks. In the absence of sufficient areawide control measures targeting ticks, arthropod repellents can prevent transmission of tick-borne diseases and remain a primary option for personal protection to reduce tick bites and tick-borne diseases in tick habitats (Carroll et. al. 2005, Piesman and Eisen 2008). However, most repellents currently being used and recommended for ticks have been developed against mosquitoes and the dramatic increase in the prevalence of tick-borne diseases compels us to evaluate repellents specifically for the prevention of tick-human contact (Bissinger and Roe 2010). In this study we evaluated a spatial repellent, d-allethrin, produced by a device designed to protect humans from mosquito bites for its effect on questing behavior of adult ticks and nymphs in the presence and absence of an attractant using a wind tunnel and an olfactometer in a laboratory setting at the Anastasia Mosquito Control District (AMCD), St. Augustine, Florida.

The first repellency test was conducted in a modular 52 cm x 52 cm x 156 cm long suction-type clear glass wind tunnel (Fig. 1) using laboratory reared male and female adult lone star ticks (*Amblyomma americanum*) obtained from National Tick Research and Education Resource (NTRER), Oklahoma State University, Stillwater, OK. Six 15 min replicates were conducted, three using between 20-40 males for each replicate and three using 20-40 females, to measure tick movement towards a lure only and similarly 6 replicates were conducted to measure tick movement towards a combined lure and repellent. The wind tunnel plenum

was marked with five 30 cm long sections to track movement of ticks from the release point (Fig. 1). The lure consisted of two BG lure cartridges (Biogents, Regensberg, Germany) and the repellent consisted of a Thermacell mosquito repellent device (Model MR300, Thermacell Repellents Inc, Bedford, MA) containing 455 mg d-allethrin in a heated paper mat (Bibbs & Xue 2016). For each lure-only replicate, the lure was placed at the upwind treatment release point (Fig. 1) and ticks were released at the downwind tick release point (Fig. 1) and allowed free movement for 15 min, after which the number of ticks in each section of the wind tunnel plenum was recorded. For each lure+repellent replicate, the procedure was the same except both the repellent device and the lure were placed at the upwind treatment release point before releasing and counting the number of ticks in each section after 15 min.

The second repellency test was conducted using a True Choice Olfactometer (Sigma Scientific, Micanopy, FL) to investigate the repellency of d-allethrin vapors produced by the Thermacell device to NTRER lone star tick adults and nymphs. Ticks were released in the acclimation chamber and their movements into the two choice chambers after 10 min observed with and without the presence of repellent: three replicates each with new sets of ticks were conducted with fresh air drawn through both choice chambers of the olfactometer, and three replicates each with new sets of ticks were conducted with fresh air drawn through one choice chamber and d-allethrin vapors from the Thermacell drawn through the other choice chamber. Adult ticks from repellent replicates and nymphs from all replicates were retrieved and stored in vials for 24 h mortality determination.

For both the wind tunnel and olfactometer tests, different numbers of ticks were used in each replicate so the numbers that had moved into each section of the wind tunnel or olfactometer were converted to percentage of the total ticks used in that particular replicate. Thus, all data are reported here as percent ticks in each section or chamber. Data from the wind tunnel test were used to determine the weighted mean distance travelled by ticks using the formula:

$$\textit{Weighted Mean Distance} = \frac{\Sigma_{i=1-5} N_i \times D_i}{\Sigma_{i=1-5} N_i}$$

Where N = number of ticks in each section i, and D = distance from end of tunnel to the mid-point of each section i, which were 15,

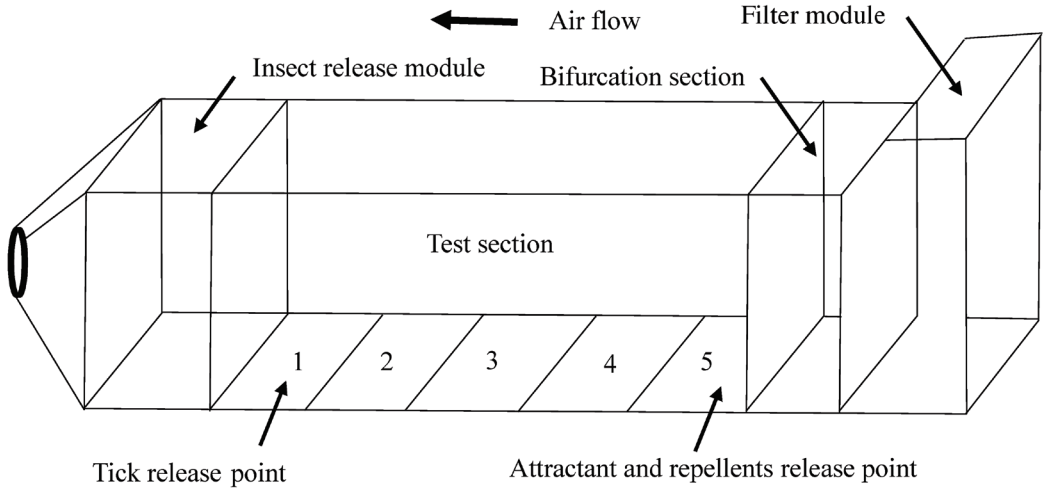

Figure 1. Diagram of wind tunnel showing tick release point and the location of the attractant and repellent release point.

Table 1. Mean percent of ticks across the 5 sections of the wind tunnel plenum test area.

Treatment	Sex	Mean number ticks	Mean percent (± SE) of ticks in each section				
			1 – 31 cm (Tick release point)	31 – 62 cm	62 – 93 cm	93 – 124 cm	124 – 156 cm (Attractant and repellant release point)
Lure	Male	19	17.7 ± 3.0	19.7 ± 5.7	18.2 ± 4.2	9.4 ± 6.6	35.0 ± 3.2
	Female	16	13.0 ± 5.2	23.0 ± 1.0	11.4 ± 6.3	17.4 ± 2.8	35.2 ± 1.2
Lure + Repellent	Male	16	43.4 ± 3.4	15.0 ± 1.6	16.2 ± 4.4	10.5 ± 3.7	14.9 ± 4.8
	Female	16	40.8 ± 4.9	16.4 ± 4.3	6.6 ± 3.4	12.2 ± 8.2	24.0 ± 4.0

45, 75, 105, and 135 cm for sections 1-5, respectively. All data were tested for normality with JMP Version 14 and found normal. Data from wind tunnel and olfactometer tests were analyzed using the analysis of variance procedure in JMP version 14 and means were evaluated using a *t*-test at 95% confidence level.

Mean tick counts in each wind tunnel section are summarized in Table 1. The analysis indicated that regardless of the presence of the repellent, males and females behaved similarly. For both sexes, there were more ticks on the upwind treatment side when only lure was present but the trend reversed and fewer ticks were present on the upwind side when repellent was present with the lure at the upwind side. Specifically, ignoring the middle section (section 3), the upwind treatment release side (sections 4 and 5) had significantly higher percentage of ticks (48.5%) than on the downwind tick release side (sections 1 and 2; 36.7%) when only lure was in place indicating ticks were attracted to the lure. However, when the d-allethrin repellent was present with the lure on the upwind treatment release side, the tick release side had a significantly higher percentage of ticks (57.8%) than the upwind treatment release side (30.8%) indicating that d-allethrin vapors repelled ticks even in the presence of lure. As shown in Fig. 2, tick movement toward the lure was significantly reduced by approximately 20 cm in the presence of the d-allethrin repellent.

Results in Table 2 from the olfactometer test show strong repellency of d-allethrin vapors to adult ticks but less repellency to nymphs. Similarly, mortality data from the olfactometer test showed that d-allethrin vapors killed 87% of adults in 24 h while mortality of nymphs was 38% and 32% for no repellent and repellent treatments, respectively.

Most lone star ticks were repelled away from d-allethrin vapors in the wind tunnel test, although the vapor did not completely prevent some ticks from moving towards the repellent. Likewise, adults—but not nymphs — were mostly repelled in the olfactometer test, in which we also observed high mortality in

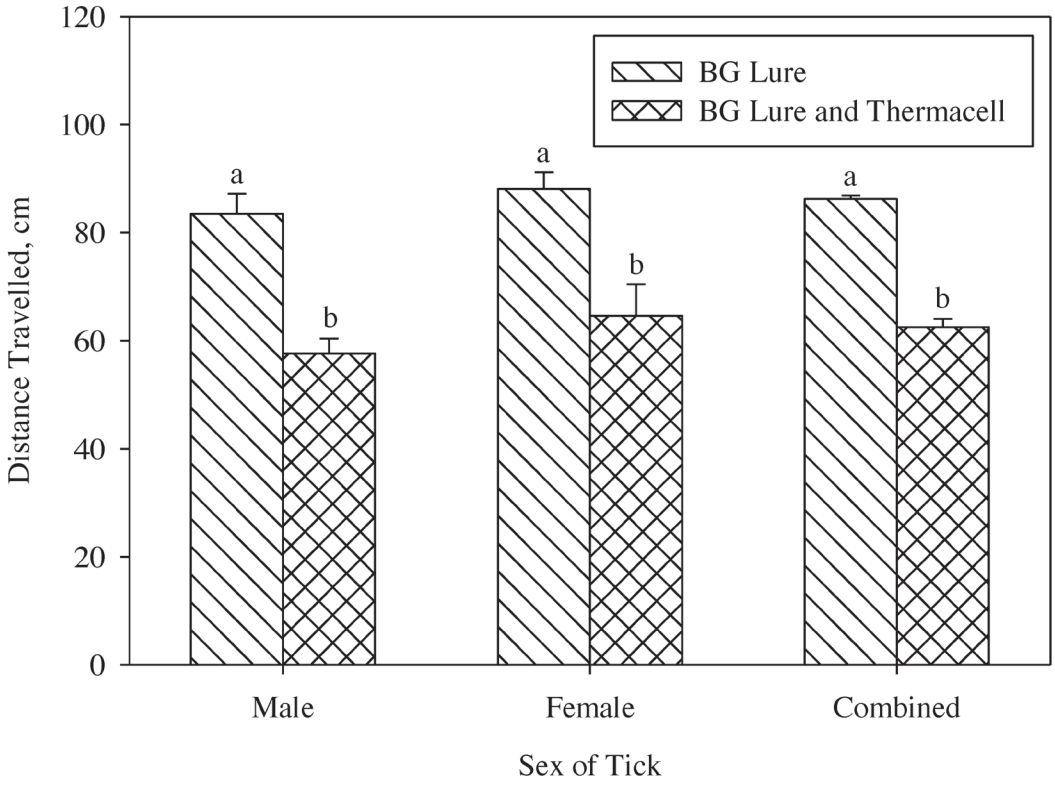

Figure 2. Comparison of weighted mean distances travelled by ticks showing significant reduction of approximately 20 cm in the presence of combined lure and d-allethrin.

adults in the presence of the d-allethrin vapor. The response of lone star ticks to d-allethrin in these tests were similar to results reported by Bibbs & Xue (2016) and indicates that the d-allethrin vapor generated by the pad could be effective as a personal tick repellent and should be further evaluated in semi-field and field conditions, and against natural populations of the lone star tick and other species.

ACKNOWLEDGMENT

We thank J. Hainze for technical assistance and review of the manuscript, Thermacell, LLC, for partial funding of the project, and J. Estaver and R. Strohschein (Sigma Scientific LLC) for providing the olfactometer. This is a research report only and the mention of commercial products is to pro-

Table 2. Mean percent of ticks across the 3 chambers of the True-Choice Olfactometer.

Treatment	Total number ticks	Mean percent (± SE) ticks in each chamber			Mortality, %
		Fresh air side	Acclimation Chamber	Repellent side	
Adults					
No Repellent	15	26.7 ± 13.3 bA	26.7 ± 6.7 aA	46.7 ± 17.6 aA	
Repellent	15	86.7 ± 6.7 aA	6.7 ± 6.7 aB	6.7 ± 6.7 aB	87
Nymphs					
No Repellent	16	20.0 ± 11.6 aA	36.7 ± 8.8 aA	43.3 ± 12.0 aA	38
Repellent	16	43.3 ± 12.0 aA	37.8 ± 2.2 aA	18.9 ± 11.6 aA	32

Different small letters in columns for adults and nymphs and different capital letters in rows indicate significant difference from ANOVA and *t*-tests at the 95 % confidence level.

vide specific information and does not imply endorsement by AMCD.

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EDITORIAL ACKNOWLEDGEMENTS

The following scientists have provided valued assistance in reviewing articles for this issue. Names followed by an asterisk (*) are individuals who have reviewed two or more manuscripts. A special thank you is given to these scientists by the editors. The editors also acknowledge S. Peper, M. Farooq, M. Sallam, V. Aryaprema, Y. Jiang, C. Bibbs, and W. Qualls as Guest Editors for the peer-review and editing process of a manuscript. Also, the editor thanks A. Hartnett for editorial assistance and proofreading.

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