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New manuscripts of articles, operational or scientific notes, annual meeting abstracts and page proofs should be sent to the Editor by e-mail attachment at xueamcd@gmail.com. The Editor will assemble the manuscripts for the assistant editor/subject editor or guest editor to conduct the peer review process.

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2021-2022 THE FMCA PRESIDENTIAL ADDRESS

Christopher Lesser
Manatee County Mosquito Control District, Palmetto, Florida

Greetings and I am happy to be asked by the Editor of the Journal of the Florida Mosquito Control Association to submit a summary of accomplishments the FMCA Board over the past year (November 2021- Nov. 2022) where I was honored, privileged to serve as the President. First, please allow to point out that no president should ever act/force their agenda from a pulpit in a singular, forceful manner but rather manage a forward-thinking agenda in manner that is supportive by the entire Board. In addition, in an ideal world, agenda aren't a function of a single person/single President but rather a series of goals that often span several years. I was happy to serve after several great FMCA presidents most notably Donnie Powers and James Clauson with all 3 collectively working together across Boards to "right-the-ship" as we collectively deemed correct. Fortunately, we all worked with Boards that were also progressively-thinking and can not readily recall a single agenda/motion that wasn't unanimously supported.

Over these three years, with the initial push by Donnie, a great amount of effort was placed into fiscal accountability and transparency. A profession, third-party bookkeeper was hired to oversee all daily financial transactions. A Treasurer (FMCA member) was added to the Bylaws to oversee and work cooperatively with the President, Executive Director and Finance Committee Chair to ensure that FMCA money was being spent appropriately. Annual professional audits were restarted. And credit cards were removed from all committees. The Finance Committee was re-structured in 2020. And a professional Executive Director was hired to manage all aspects of the FMCA (finances, membership services, and annual meetings). But growth sometimes has pain and sometimes, some members might be resistant to change. Unfortunately, we all experienced some of this resistance. Despite our best efforts, Donnie's first attempt to complete a fiscal audit of the 2019 Calendar Year was unsuccessful due to many gaps, missing accounts and incomplete records. Fast forward to 2022: Due to the focus on fiscal accountability by multiple people across several Boards, the FMCA is now in excellent fiscal health with total available cash of $568,024 and an additional $69,519 in the FMCA Foundation (as of November 2022). The professional audit of the 2021 FMCA accounts found no findings or recommendations.

Moving Forward: Dennis Moore (past Executive Director Pasco County MCD) was hired in January 2022 as the new Editor-in-Chief of Wing Beats. In a rather short 12 months, Dennis has done a remarkable job in improving the professionalism of an already great publication by actively recruiting articles of interest, engaging with new advertisers, improving the "look" and layout of Wing Beats. Congratulations to Dennis and his team of editors who now boast a world-wide circulation of 3,700 individuals in a quarterly magazine that is enjoyed by many. In addition, the FMCA Board created (and approved in Bylaws) a new FMCA Young Professional Committee lead by Chair Tarolyn Frisbie (Citrus MCD) & Casey Crockett (ADAPCO - advisor). Tarolyn and Casey hosted a very successful inaugural YP series of meetings/get-togethers at the 2022 FMCA Annual meeting in Palm Coast. While on the topic of new committees, the Board approved the creation of the UAV Committee being led and managed by Peter Brake. The intent of this committee is to keep members aware of new regulations impacting this developing field as well as to education on new treads/developments.

More achievements: In 2022, the FMCA & AMCA re-established a working agreement on Wing Beats describing the responsibilities on this shared publication. The FMCA, and specifically the Legislative Committee led by Keira Lucas (Collier MCD) continues to work with Chris Lyon/LL&W on new drone/UAV regulations and how this new State law managing drones impacts mosquito control districts. In addition, Keira continues to work with LL&W and OPPAGA on Special District accountability review as required by FL Legislature in 2021. For reference, the Office of Program Policy Analysis and Government

Accountability (OPPAGA) is a research arm of the Florida Legislature and is responsible for performance reviews of the 15 independent special taxing district Mosquito Control programs in Florida. These OPPAGGA reviews should be completed by September 2023 with a final report being submitted to the State Legislature shortly thereafter. In response to the mandated OPPAGGA review, the Board hired a political-public relations firm based in Tallahassee to defend/promote/provide education on/of the entire mosquito control industry in Florida. This work is now ongoing with oversight and management provided by several FMCA members. Finally, the FMCA Board added a Liasson to 5 major committees (Aerial, Dodd, Finance, Legislative, YP) to ensure a unified that each of these 5 major committees is working towards a common agenda parallel with the FMCA Board.

It has been an absolute please working with 4 FMCA Boards over the past years and I believe that great achievements have been made to advance the Association's goals. Some of these items have been easy and common-sense changes with universal support; others have been more challenging and likely ruffled a few feathers and for this, I sincerely apologize. The FMCA is a great Association with many great individuals who want to work together to achieve a common goal. And, I thank you for allowing me to serve as the FMCA President in 2022.

In addition, we are celebrating the FMCA's 100 year anniversary.

THE RISE AND FALL OF ST. LOUIS ENCEPHALITIS VIRUS IN FLORIDA

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ABSTRACT

St. Louis encephalitis virus has had a fascinating history in Florida. The virus was introduced into the Miami area in the early 1950s. This introduction resulted in two human outbreaks, one in 1952 and a second in 1958. Three urban SLE epidemics were reported in the Tampa Bay area in 1959, 1961, and 1962. The virus virtually disappeared from the state until 1977 when a widespread rural SLE epidemic was reported in 20 Florida counties with 110 confirmed human cases. An almost identical rural SLE epidemic was reported in 1990 when 226 human SLE cases were reported in 28 Florida counties. Following the introduction of West Nile virus into Florida in 2001, reports of SLEV transmission to sentinel chickens and humans decreased dramatically. Except for a 2014 focal outbreak of two SLE cases in Duval County, only sporadic transmission of SLEV to humans and sentinel chickens was reported in the state between 1998 and 2022.

Key words: St. Louis encephalitis virus, arbovirus, mosquito-borne virus, sentinel chicken arboviral surveillance

THE INTRODUCTION OF SLEV INTO FLORIDA

The first isolation of St. Louis encephalitis virus (SLEV, genus *Flavivirus*) in Florida was from a 1952 human case in Miami (Bond et al. 1963). Six years later in 1958, the first multiple-case outbreak of SLE was reported in the same geographic region of Miami as the 1952 case when five humans were diagnosed with SLE (Ehrenkranz et al. 1963). Onset dates for these cases ranged from September 23 through December 29, 1958. Ages of the cases ranged from 32 to 77 and four were female. The 1958 Miami SLE outbreak occurred coincidently with human SLE epidemics in the Caribbean Basin and Central America. Human cases were reported in Panama in February, Jamaica in August, and Trinidad and Panama in September (Ehrenkranz et al. 1963).

In 1957 sporadic SLEV activity was detected in the Tampa Bay area when the virus was isolated from cerebrospinal fluid of a man suffering from mild aseptic meningitis (Brody and Murray 1957). This case foreshadowed the Tampa Bay area urban SLE epidemics described below.

THE URBAN SLE EPIDEMICS OF 1959, 1961, AND 1962

The virus reemerged as a series of human epidemics in the Tampa Bay area in 1959, 1961, and 1962. In 1959, a focal outbreak of human neurologic disease was reported in Pinellas County, Florida (Figure 1). Between August and November 1959, 68 cases of neurologic illness (19 aseptic meningitis and 49 clinical encephalitis (48 SLE and one eastern equine encephalitis virus)) and five deaths were reported. The epidemic peak occurred in late October. Intense vector control efforts began on 10/26/1959 and 10/30/1959, well after most of the cases were infected. Most cases in this outbreak were reported in urban St. Petersburg (southern Pinellas County) (Bond et al. 1963). A follow-up serosurvey conducted in January 1960 documented an estimated 5,000 subclinical cases of SLE for a subclinical to clinical ratio of 68:1 for the 1959 outbreak. The known urban vector of SLEV, *Culex quinquefasciatus*, was virtually absent during this epidemic while the rural vector, *Cx. nigripalpus* was far more abundant (Bond et al. 1963). The human SLE epidemic vector was not documented for the 1959 outbreak.

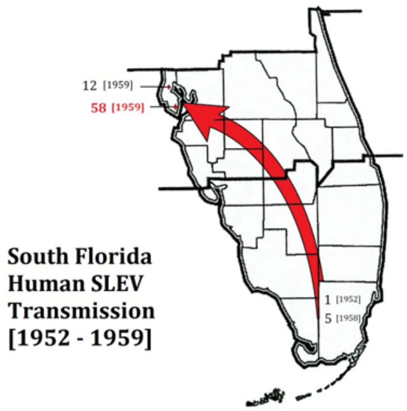

Figure 1. The early years of human SLEV transmission in Florida.

There was no evidence of SLEV transmission in the Tampa Bay region in 1960 (Figure 2). This is interesting considering the 1959, 1961, and 1962 sequence of human SLE epidemics in the region. One possible explanation for this is that Hurricane Donna that passed through the Tampa Bay area as a Category 2 hurricane on September 10th, 1960. Hurricanes tend to disrupt vector-borne disease transmission cycles because they disperse mosquito vectors and avian amplification host and disrupt mosquito and avian breeding cycles.

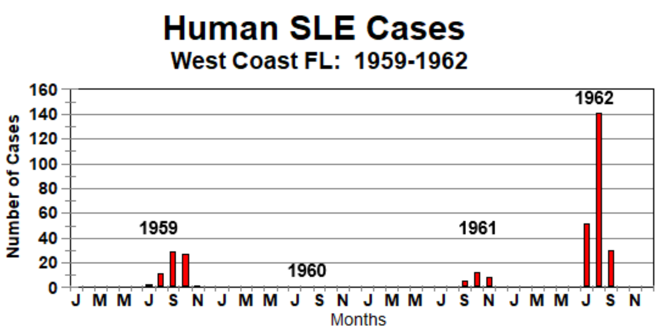

Figure 2. The temporal distribution of human SLE cases during the Tampa Bay area epidemics of 1959, 1961, and 1962.

The second of three SLE epidemics occurred in 1961 when 25 cases and seven deaths were reported in the Tampa Bay area (Chamberlain 1980). Unlike 1959 when the cases were restricted to southern Pinellas County, the cases in 1961 were reported in three adjacent counties: Pinellas (9 cases), Manatee (10 cases), and Sarasota (6 cases) (Waters et al. 1963). Onset dates for these cases ranged from 10/3/61 through 12/17/61. The peak transmission of human SLEV was reported in mid-November. The outbreak began in southern Pinellas County in early October.

This outbreak was unusual for three reasons. First, the case-fatality ratio of 28% was unusually high. Second, the outbreak was unusually short, lasting only seven weeks. Finally, the onset of disease was two months later than normal. Mosquito control in all three counties was discontinued in August because of a lack of mosquito biting complaints, no rainfall 40 days prior to the beginning of the outbreak, and low mosquito catches. However, all patients interviewed remembered mosquito bites prior to disease onset (Waters et al. 1963).

The final and most extensive SLE epidemic, in terms of the number of cases and the spatial distribution of the cases, in the Tampa Bay area occurred in 1962 (Figure 2). From mid-July through mid-October, 222 confirmed cases and 43 deaths (case-fatality ratio of 19.4%) were reported in Hillsborough (20 cases), Manatee (16), Pinellas (171), and Sarasota (15) Counties. The epidemic began in urban St. Petersburg in mid-July and spread north, east, and south into Clearwater (Pinellas County), Tampa (Hillsborough County), Bradenton (Manatee County), and Sarasota (Sarasota County) in mid-August with the last case reported in Hillsborough County during the second week of October (Bond et al. 1965).

It was during this epidemic that *Culex nigripalpus* Theobald was incriminated as the SLE epidemic vector in Florida. Forty-two SLEV isolates were made from 64,000 mosquitoes collected in chicken-baited miniature light traps. Most (75%) of the mosquitoes were *Cx. nigripalpus* from which 40 SLEV isolates were made. An isolate of SLEV was made from one pool of *Anopheles crucians* Wiedemann and from one pool of an unidentified *Culex* (*Melanoconion*) spp. (Taylor 1969). In addition to the SLEV isolates from mosquito pools, SLEV isolates were also made from four human SLE cases in 1962.

THE RURAL SLE EPIDEMICS OF 1977 AND 1990

No human SLE cases were reported in Florida from 1962-1976 except for 1969 when three human infections were reported in Polk County with onset dates in late August (2 cases) and early September (1). In 1977 a dramatic shift in the SLE epidemic transmission pattern was observed in Florida. Prior to 1977, SLE epidemics were primarily urban with transmission foci in Miami, St. Petersburg, Clearwater, Tampa, Bradenton, and Sarasota. The 1977 SLE epidemic in Florida marked a shift from urban transmission to rural transmission (Day 2001). The index case (first person infected) for the 1977 epidemic lived in Fellsmere, Florida (Indian River County). The infected individual had an onset date of August 8, 1977. The approximate infection date for this individual was July 30, 1977. What followed was an epidemic of 110 confirmed human SLE cases and 8 deaths (7.3% case-fatality ratio) in 20 south-central Florida Counties (Figure 3). The onset date for the final case in the epidemic was 12/5/77, in an outbreak that was more widespread than any previously reported SLE epidemic in Florida (Nelson et al. 1983).

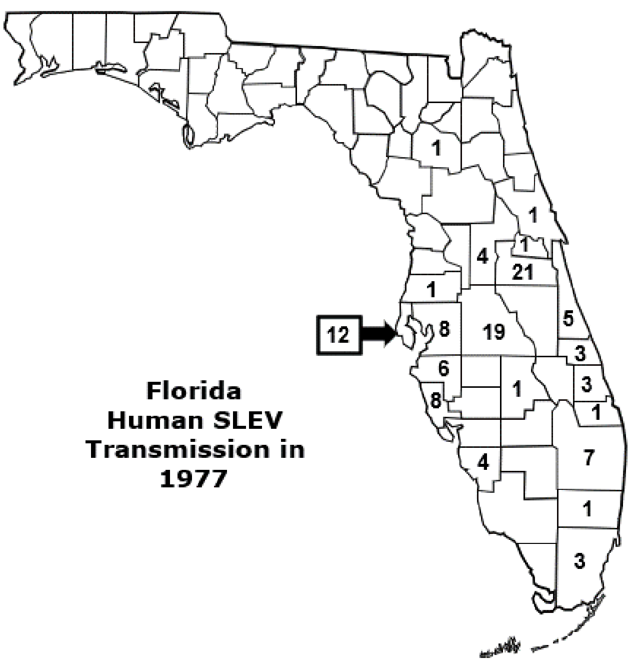

Figure 3. The spatial distribution of 110 human SLE cases in 20 Florida counties during 1977.

Sporadic human SLE cases were reported in South Florida between 1979 and 1985 with the notable exception of 1980 when a cluster of five human SLE cases was reported in Okaloosa County in the Florida Panhandle. The transmission of SLEV to humans in the Florida Panhandle is unusual with only six confirmed SLE cases reported between 1952 and 2022. The suspected epidemic vector for this outbreak was *Culex quinquefasciatus* Say (McCaig et al. 1994).

In 1990, a second widespread rural SLE epidemic that was remarkably similar to the 1977 outbreak was reported in South Florida (Meehan et al. 2000). The index case for the 1990 SLE epidemic was a 22-year-old male who, like the index case for the 1977 SLE epidemic, lived in Fellsmere, Florida (Indian River County). The onset date for the 1990 index case was July 28, 1990, with an approximate infection date of July 19. The 1990 SLE epidemic continued for 25 weeks in 28 Florida counties (Figure 4) where 226 laboratory-confirmed human cases and 11 deaths (case-fatality ratio of 4.9%) were reported. The final human case was a 72-year-old male from Polk County with an onset date of 1/8/90 and an approximate infection date of 12/30/90 (Day 2001).

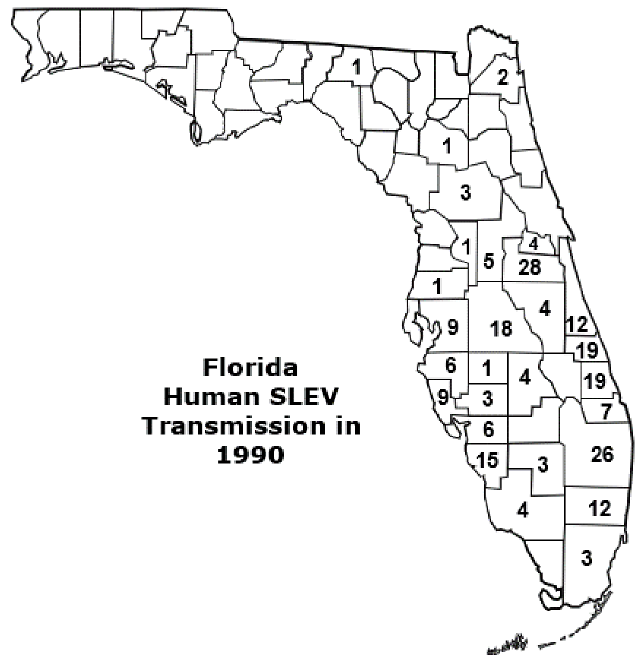

Figure 4. The spatial distribution of 226 human SLE cases in 28 Florida counties during 1990.

The 1990 Florida SLE epidemic marked the final reported epidemic of this virus in Florida. Between 1991 and 1999 focal (1993) and sporadic (1997) outbreaks of SLE in humans were reported in the state (Day and Stark 2000). However, no significant transmission of SLEV has been reported in Florida since 1990. Exact reasons for this decline are not known and some possible explanations are discussed below.

POSSIBLE REASONS FOR THE URBAN TO RURAL SHIFT AND THE DECLINE OF SLEV IN FLORIDA

The shift from an urban SLE transmission pattern (1958, 1959, 1961, and 1962) to a rural transmission pattern (1977 and 1990) was dramatic. One consistent factor between both types of transmission was the epidemic vector, *Cx. nigripalpus* (Dow et al. 1964, Nelson et al. 1983, Shroyer 1991). *Culex nigripalpus* is a subtropical mosquito that is extremely abundant in South Florida. The species is most abundant during the summer rainy season and population peaks follow heavy rainfall events (Day and Curtis 1994). This species is an opportunistic blood feeder that oviposits in a wide variety of aquatic habitats found in urban and rural habitats (Provost 1969). The rural SLE epidemics of 1977 and 1990 mimicked past SLE epidemics in the western United States where *Cx. tarsalis* Coquillett was the primary epidemic vector and epidemics were associated with agricultural irrigation practices (Hammon 1941, Sciple et al. 1968). It is significant that a majority of human SLE cases in 1977 and 1990 occurred in regions of Florida where citrus farming and its associated irrigation patterns were abundant (Day and Curtis 1994).

The marked decline in the transmission of SLEV in Florida from 1998 to 2022 may have several explanations. Environmental factors may be important. All six of the Florida SLE epidemics (1958, 1959, 1961, 1962, 1977, and 1990) were preceded by severe winter freezes (Day and Shaman 2009). There have been no major freezes in South Florida since the Christmas freeze of 1989.

West Nile virus (WNV, genus *Flavivirus*) was first detected in Florida in 2001 (Blackmore et al. 2003). West Nile virus and SLEV are serologically related flaviviruses that circulate between *Culex* spp. mosquitoes and wild avian amplification hosts in well-defined transmission foci. Reports of a decline in transmission of SLEV in Florida and California suggest that there may be an immunological interaction between WNV and SLEV in wild avian amplification hosts reducing the transmission of SLEV in areas where the viruses overlap. It has been proposed that cross-neutralization in avian amplification hosts may explain the reduction of SLEV transmission after the introduction of WNV (Reisen et al. 2003, Fang and Reisen 2006, Reisen et al. 2008). However, Maharaj et al. (2018) have presented genetic evidence that, along with the co-circulation of WNV and SLEV in Arizona in 2015 (Yang et al. 2028), demonstrates pre-existing cross-neutralization immunity in avian amplification host populations does not eliminate the co-circulation of these two viruses.

There has been speculation since 1952 about how SLEV entered Florida and how the virus is dispersed around the state. In 1963 Ehrenkranz et al. proposed the possibility that migrant birds using the Mississippi and Atlantic flyways may serve as a means of SLEV introduction into Florida. Large numbers of migrating birds travel through Florida in the autumn during their southward migration and return through Florida in the spring during their northern migration. Introduction and dispersal of SLEV during both migrations is possible. Bond et al. (1963) suggest that migrant birds, along with their periodic delays due to environmental conditions (the migration pilling-up effect) may be an important factor in the re-introduction and dispersal of SLEV in Florida. Likewise, Jennings et al. (1963) maintain that migrant birds were important to SLEV transmission in the Tampa Bay area in 1959 and 1961. They observed that areas surrounding most of the known human cases were heavily vegetated and contained ideal roosting sites, not only for resident wild birds, but also for migrants.

THE FUTURE OF SLEV IN FLORIDA

It is evident that the transmission of SLEV in Florida has declined since 2000. The Florida Sentinel Chicken Arboviral Surveillance Program has been in place since 1978 (Day and Lewis 1992). During the lifetime of this program the Florida Department of Health has issued weekly, monthly, and annual summaries concerning arboviral transmission within the state including summaries for the sentinel chicken program. For the 23-year period from 1978 through 2000 an annual average of 173 SLEV antibody-positive isolates were made from sentinel chickens in Florida. In contrast, during the 22-year period from 2001 to 2022 an annual average of 24 SLEV antibody-positive isolates were made. Similar post-WNV declines in SLEV transmission were reported in Arizona and California (White et al. 2016).

It appears that the post-WNV transmission pattern of SLEV in Florida will be one of focal reintroduction, probably by migrating birds. This type of viral reintroduction and transmission has been reported in Arizona and California (White et al. 2016). The reintroduction and transmission of SLEV in Florida since 2001 has been focal. For example, in 2014 two human SLE cases were reported in Duval County during a year when 106 SLEV antibody-positive sentinel chickens were reported in 13 Florida counties. Most (84, 79%) of the antibody isolates were from chickens maintained in the central Florida peninsula.

Likely, the future of SLEV in Florida will center on migrating birds, local amplification of reintroduced SLEV in resident birds, and, in rare cases, infection of vector mosquitoes to a level where viral spillover to humans is possible. The 2022 arboviral transmission season in Florida illustrates this. During 2022, 19 SLEV antibody-positive sentinel chickens were reported in 11 Florida counties (Figure 5). One positive sentinel chicken was reported in Okaloosa County in March and likely represents the reintroduction of SLEV into Okaloosa County during spring migration. There were no antibody-positive sentinel chickens reported in Florida during April-June, a period when amplification is usually reported in breeding resident bird populations (Day

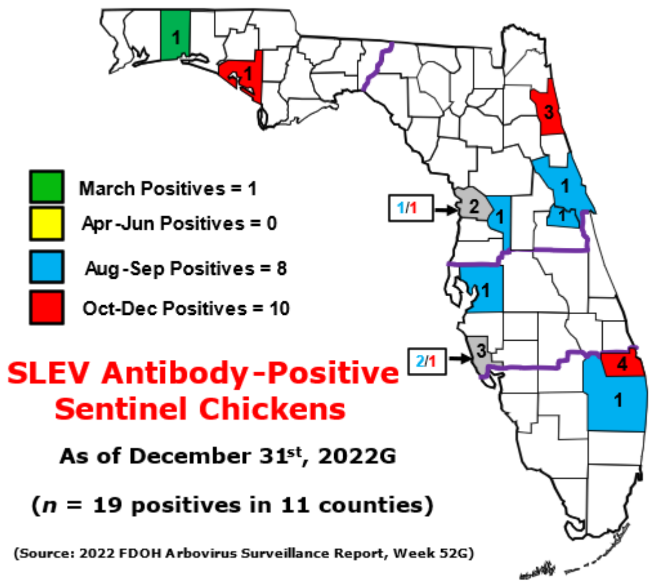

Figure 5. The spatial and temporal distribution of 19 SLEV antibody-positive sentinel chickens in 11 Florida counties during 2022.

1999). Eighteen SLEV antibody-positive sentinel chickens were reported from August-December, a period that corresponds to fall migration in Florida. Likely, large rural SLE epidemics are probably a thing of the past in Florida. Future SLE outbreaks will likely be urban and restricted to focal introductions by migrating birds followed by local amplification and spillover of SLEV to humans in small transmission foci.

ACKNOWLEDGMENTS

This analysis would not be possible without the tireless efforts of multiple agencies across Florida. At least 27 Florida agencies collect serum samples from sentinel chickens each week and mail them to the Florida Department of Health Tampa Branch Laboratory for analysis, compilation, and reporting. Data are summarized by researchers at the Florida Department of Health in Tallahassee and reported weekly as the Florida Arbovirus Surveillance Report. Contributors to weekly summaries and other reports include: Andrea Morrison, Rebecca Zimler, and Danielle Stanek, Bureau of Epidemiology; Lea Heberlein-Larson, Alexis LaCrue, Maribel Castaneda, and Amanda Davis, DOH Bureau of Public Health Laboratories; Carina Blackmore, DOH Division of Disease Control and Health Protection; Reddy Bommineni, Florida Department of Agriculture and Consumer Services, Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL.

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EVALUATION ON THE ACTIVITY AND EFFICACY OF OMNIPRENE™ 20CS AGAINST *AEDES*, *ANOPHELES* AND *CULEX* MOSQUITOES IN OUTDOOR MICROCOSMS

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Subject Editor: Whitney A. Qualls

ABSTRACT

Mosquito control by pesticides remains the main intervention to reduce the burden of mosquito nuisance and mosquito-borne diseases. In mosquito control, larviciding by application of environmentally friendly larvicides has been one of the routine operations. Larvicides based on microbial agents and insect growth regulators (IGRs) account for most of the available products. Ones that are formulated using S-methoprene ranged from microencapsulated concentrate (or capsule suspension - CS), various granules, and pellets to briquets for different habitats. The CS formulation has been among the traditional products based on S-methoprene due to its advantages of easy application, fast action, and even area coverage. The current paper evaluated a newly developed CS formulation OmniPrene 20CS™ containing 20% S-methoprene against mosquitoes of public health importance. High inhibition of emergence activity was indicated against the test species, and *Aedes* and *Anopheles* mosquitoes showed higher susceptibility than *Culex* in laboratory bioassays. The performance under field conditions exceeded the label specification of the currently available products with the same application pattern. Over the 21-day evaluation period, the control levels ranged 86.4-100%, 89.8-100% and 79.8-100% against *Aedes aegypti*, *Anopheles hermsi* and *Culex quinquefasciatus* respectively when it was applied at the intended label dose of 54.8-73.1 mL/ha. This product is expected to be used as one of the viable tools along with other available products to combat mosquitoes of public health concerns.

Key Words: S-methoprene, OmniPrene™, mosquitoes, microcosm, field efficacy

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INTRODUCTION

To reduce the impact of mosquitoes and mosquito-borne diseases on public health and well-being, sustainable mosquito control remains one of the main interventions. In mosquito control operations, combating the aquatic immature stages is more feasible and cost-effective by biorational larvicides based on microbials (Antonio-Nkondjio et al. 2018, Derua et al. 2019) and insect growth regulators (IGRs). Due to high cost of research, development and registration, strict regulations, narrow market niches and resistance development, available larvicides are very limited. To meet the need of safe and effective mosquito control products due to existing, emerging, and resurging mosquitoes and mosquito-borne diseases (Gratz 1999, Chala and Hamde 2021), innovated formulations based on available active ingredients and searching for novel active ingredients are equally viable solutions.

S-methoprene, a synthesized chiral form out of the early mixture of R- and S- enantiomers, is the only IGR that is recognized as a biopesticide by US Environmental Protection Agency (US EPA 2021), and has been playing a crucial role since the early 1970s to control insects of economic importance, mainly mosquitoes, dipteral flies, stored product pests, etc. (Henrick 2007, Su 2018). Numerous products from microencapsulated concentrate (or capsule suspension – CS), emulsifiable concentrate (EC), several granules, pellets and briquets have been developed and applied to combat numerous target species. As to the CS and EC, the advantages have been recognized such as relatively fast release of active ingredient, reduced sublethal exposure risk as compared with slow-release long residual formulations, and minimal persistence in the environment. Along with the availability of the CS formulations under another trademark (Central Life Sciences 2022), the current paper is aimed to evaluate a new product, OmniPrene™ 20CS containing 20% S-methoprene, that is formulated with innovated proprietary microencapsulation technologies, against *Aedes*, *Anopheles* and *Culex* species. High inhibition of emergence activity was indicated in laboratory bioassay, and the performance under field conditions exceeded the label specification of the currently available counterpart products with the same application pattern.

MATERIAL AND METHODS

Test Materials. The technical S-methoprene (US EPA 73487-1) was provided by Synergetica International Inc. (Marlboro NJ, USA). The sample had a Lot# MT08-218 and the purity was 98.06% by high performance liquid chromatography (HPLC). The OmniPrene 20CS (lot# SC-L210810), an innovated CS formulation containing 20% S-methoprene (US EPA registration pending 73487-3), was provided by the same supplier.

Mosquitoes. The yellow fever mosquito *Aedes aegypti* L. was supplied by Benzon Research (Carlisle, PA) from its long-term susceptible colony. The southern California malaria mosquito *Anopheles hermsi* Barr & Guptavanij originated from field-collected larvae and host-seeking female adults from southern California. The southern house mosquito *Cx. quinquefasciatus* Say was from a long-term in-house susceptible colony. The late 4th instar larvae, that were about to pupate, were used in laboratory bioassays and field efficacy evaluations.

Laboratory bioassay. Laboratory bioassay was conducted according to the previously published protocols (Su et al. 2018) against all test species to validate the quality of the test materials prior to field evaluation. The technical S-methoprene was dissolved in and serially diluted by ACS pure acetone (Cole Parmer, Vernon Hills, IL). The OmniPrene 20CS was diluted in deionized water. In the biosassys, 5 concentrations (0.025, 0.5, 2.5, 10, and 25 ppb) within the concentration range resulting in approximately 5–95% mortality, plus untreated control (UTC) were used, with 3 replicates at each concentration and UTC. Each aliquot with the appropriate dilution in the amount of 100 to 500 µL was added to 100 mL tap water, where the volume increase was negligible. In technical S-methoprene, the acetone in the bioassay water evaporated quickly without an observable impact on the Styrofoam polystyrene cup as was demonstrated with the test subject and the UTC that had the solvent only. For each replicate, 25 late instar larvae were placed in 100 mL tap water in a 120-mL disposable cup. A small piece (approximately 100 mg) of rabbit pellets was added to each bioassay cup to have slow release of the nutrients to support larval growth to pupation. In mosquito species, late instar larvae are more susceptible than the early instar larvae to external JHAs such as S-methoprene (Noguchi and Ohtaki 1974). Young larvae have low susceptibility to JHAs due to high internal juvenile hormone. The bioassay using young larvae would underestimate the activity of JHAs, take longer to complete or end with inconclusive results because the peak concentration of JHAs and susceptibility window of larvae miss each other upon degradation of active ingredient and larval growth. Therefore, late instar larvae, sometimes called pupating larvae were used in the current studies to ensure the maximized and synchronized inhibition of emergence in the laboratory bioassays. Bioassays were conducted at 27.0-29.0°C. The mortality was recorded when all exposed individuals died or emerged as adults. Only those that fully separated from their pupal exuviae were considered to have successful emerged.

Field evaluation. *Test Facility and Treatment* Test was carried out in outdoor microcosms that were black color plastic tubs located at a protected, semi-shaded area with trees and other vegetation in suburban Riverside, California. The microcosms measured L0.81 x W0.51 x D0.41 meter. A 1.3-cm deep substrate of sandy loam soil was added to each microcosm to simulate the natural habitat. The soil was collected from a nearby orchard, where there were no known recent records of pesticide and herbicide applications. Water depth was maintained at 0.305 meter for each microcosm (approximately 118 liters) with a surface area of 0.41 square meter. Rabbit food pellets were added to each microcosm at 0.005% (about 6 g each microcosm) as larval food and organic enrichment, after flooding and on a weekly basis thereafter. A minimum-maximum thermometer was put at bottom of one tub that was in the middle of the microcosm layout to monitor water temperature range during each sampling interval. The microcosms were covered by a window screen (1.4 mm) to prevent oviposition by the natural mosquito populations (mostly *Culex* spp.) during non-sampling times. Five replicates (tubs) were made of each treatment and UTC.

The treatment was made on day 5 post-flooding, when enrichment was well fermented, and sediment had settled out. The application doses were 54.8 and 73.1 mL/ha as recommended on intended product label, equivalent to 2.125 µL and 2.834 µL of original product per microcosm. The product was diluted by adding 500 µL product to 499.5 mL of deionized water resulting in 1000-time dilutions for ease of application and even coverage. Each microcosm was treated by pipetting 2.125 mL and 2.834 mL of the diluted product over the surface.

Efficacy assessment The previously insectary-reared late 4th instar larvae of the three test species were introduced on day 0, 2, 4, 7, 14 and 21 post-treatment, where day 0 referred to day of treatment. The late instar larvae were used here for the same reason as described previously. To collect 25 pupae after introduction on one occasion, 50 larvae were introduced to each sentinel cage in each microcosm for each of the three test species. The sentinel cage was made of 946.4 mL square plastic tub, 5.1 x 5.1 cm square window was made in the center of each side, the window was covered by 0.30 mm mesh to allow water running through freely but to retain the larvae and pupae. The same window was also made in the center of the lid to ensure ventilation and to keep debris from falling into the cage. A plastic foam belt was attached underneath the rim of the cage as a floatation device. Twenty-five (25) pupae were collected from each of five (5) replicated microcosms 2-3 days (varied from cage to cage) after larval introduction when most introduced larvae had pupated. Collected pupae were held in 100-mL of water from the same microcosm in a 177.4-mL Styrofoam cup. In total, 125 pupae from each treatment or UTC were considered an adequate number for percentage calculation of emergence inhibition. After a one-time pupal collection, the remaining larvae and pupae in the sentinel cages were rinsed to the ground and disposed of. Cups with pupae were covered by a plastic dome with a 2.5-cm diameter screened top (1.4 mm mesh) and emerged adults were confined under this dome. Around day 3-4 after pupal collection, when all pupae died or emerged, surviving adults were released to a 30.5 x 30.5 x 30.5 cm screened mosquito cage, and only mosquitoes that were free from their pupal exuviae were counted and considered as successfully emerged. Observation on inhibition of emergence was conducted at 27.8-29.4°C. Adults released to the mosquito cage during result reading died later due to sugar and water deprivation and were disposed of properly later.

DATA ANALYSIS

Concentration−response data in laboratory bioassays were corrected by Abbott formula to factor the mortality in UTCs (Abbott 1925), then analyzed using POLO Plus (Robertson et al. 2006) for probit analysis to calculate the concentrations causing 10%, 50% and 90% inhibition of emergence (IE) and their 95% confidence intervals (95% CIs). Significant differences in IE were indicated by separate 95% CIs (Su et al. 2018). In field test, the mean IE% for UTC and each treatment on each sampling day was calculated: IE% = 1- (number of successfully emerged adults/total number of pupae collected). The IE% between the doses and among the sampling intervals were analyzed by *Chi* square test after correction by Abbott formula for the significance at $\chi^2 > 3.84$, $p < 0.05$ and $\chi^2 > 6.63$, $p < 0.01$ levels.

RESULTS

Laboratory bioassay. Concentration-response was established in the concurrent bioassays using technical S-methoprene and OmniPrene 20CS against all test species, with low mortality in all UTCs (5.3-9.3%). High

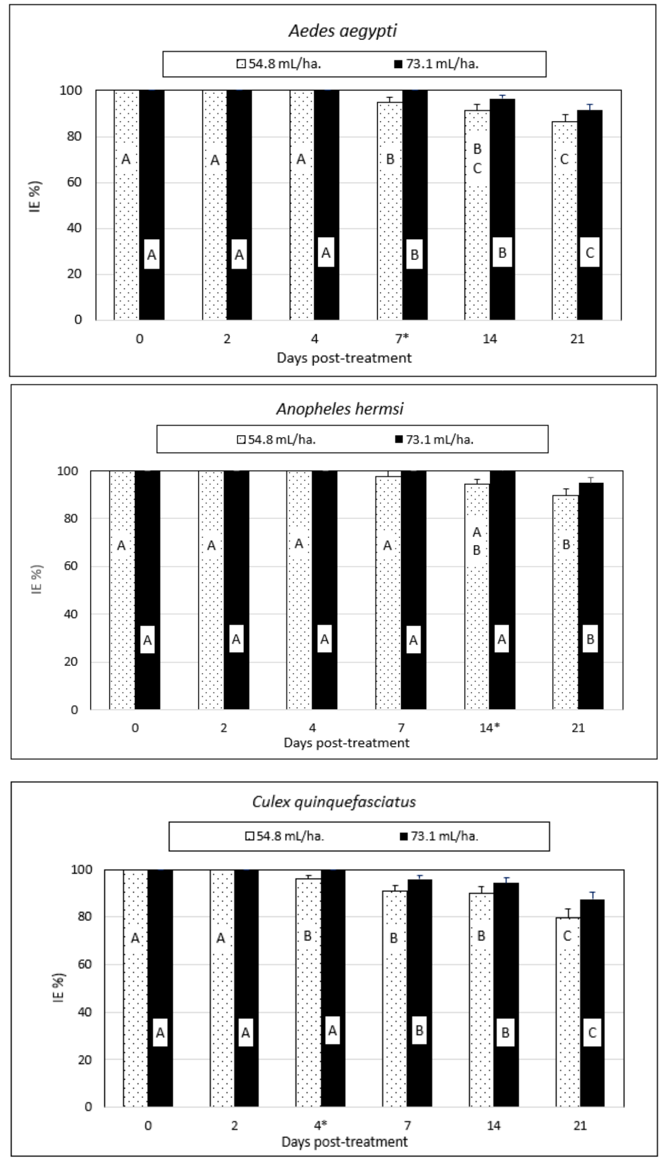

Figure. 1. Corrected inhibition of emergence (IE) against *Aedes aegypti* (Top), *Anopheles hermsi* (Middle) and *Culex quinquefasciatus* (Bottom) by OmniPrene 20CS in microcosms. The significance between two doses was indicated by "*" associated with a given sampling day. The significance in efficacy decline over time was indicated by different capital letters inside the columns for each of two doses ($\chi^2 > 3.84$, $p < 0.05$; $\chi^2 > 6.63$, $p < 0.01$).

activity of inhibition of adult emergence was indicated in all test species after data correction by Abbott formula. By comparing the IE_{10}, IE_{50} and IE_{90}, *An. hermsi* appeared more susceptible than *Ae. aegypti*, but the differences did not reach the significant levels, as their 95% CIs overlapped. However, both *Ae. aegypti* and *An. hermsi* were more susceptible than *Cx. quinquefasciatus* as indicated by their separate 95% CIs. There were no significant differences between the technical S-methoprene and the formulated product OmniPrene 20CS against all test

Table 1. Laboratory bioassays on technical S-methoprene and OmniPreneTM 20CS containing 20% S-methoprene against the test species*.

Products	IE_{10} (ppb) (95% CI)	IE_{50} (ppb) (95% CI)	IE_{90} (ppb) (95% CI)
		Aedes aegypti†	
Technical S-methoprene	**0.059** (0.032-0.123)	**0.932** (0.699-1.731)	**9.532** (6.512-13.980)
OmniPrene 20CS	**0.060** (0.036-0.152)	**0.831** (0.389-1.342)	**8.881** (6.211-12.363)
		Anopheles hermsi†	
Technical S-methoprene	**0.014** (0.009-0.039)	**0.459** (0.245-1.034)	**4.331** (2.834-7.945)
OmniPrene 20CS	**0.022** (0.010-0.044)	**0.434** (0.255-0.906)	**4.692** (2.912-6.883)
		Culex quinquefasciatus†	
Technical S-methoprene	**0.189** (0.137-0.432)	**2.408** (1.855-4.012)	**17.587** (14.344-30.321)
OmniPrene 20CS	**0.192** (0.156-0.445)	**2.138** (1.357-3.122)	**16.823** (13.832-32.345)

*Mortality data was corrected using Abbott formula (Abbott 1925) to factor the mortality in UTCs (5.3-9.3%) before probit analysis.
† The susceptibility of *Ae. aegypti* and *An. hermsi* was significantly higher than that of *Cx. quinquefasciatus* as indicated by separate 95% confidence intervals (CIs).

species at three (3) IE levels as shown by overlapped 95% CIs (Table 1). Mortality occurred in an overall pattern of incomplete adult emergence, dead intermediate form of larvae and pupae (also known as "puparvae") (Su et al. 2020), dead pupae and dead larvae, upon the increases of S-methoprene concentrations.

Field evaluation. *Efficacy during 21-day evaluation* The mortality as represented by dead pupae and incompletely emerged adults were low at all UTCs, ranging 2.4-6.4% in the three test species. The corrected efficacy levels were as follows. Against *Ae. aegypti*, the low dose 54.8 mL/ha. provided 86.4 – 100% control, while the efficacy increased to 91.5 – 100% at the high dose 73.1 mL/ha. The IE was significantly higher at the high dose than that at the low dose on day 7 (χ^2 = 6.15, $p < 0.05$). The efficacy at the low dose declined initially on day 7 (χ^2 = 6.15, $p < 0.05$), and further on day 21 (χ^2 = 5.79, $p < 0.05$). The efficacy decline at the high dose was delayed to day 14 and 21 (χ^2 = 4.07-5.79, $p < 0.05$). Against *An. hermsi*, the low and high doses resulted 89.8-100% and 94.9-100% inhibition of emergence, respectively. The high dose outperformed the low dose on day 14 (χ^2 = 7.20, $p < 0.01$). The efficacy declined on day 21 at both doses (χ^2 = 6.15-6.68, p < 0.05-0.01). Lastly, the efficacy against *Cx. quinquefasciatus* was slightly lower, being 79.8-100% for the low dose and 87.4-100% for the high dose. The high dose was significantly more efficacious than the low dose on day 4 (χ^2 = 5.10, $p < 0.05$). The efficacy declined initially on day 4 (χ^2 = 5.10, $p < 0.05$) and further on day 21 (χ^2 = 5.36, $p < 0.05$) at the low dose, this initial decline was delayed to day 7 (χ^2 = 5.10, $p < 0.05$) and followed by further decline on day 21 at the high dose (χ^2 = 3.88, $p < 0.05$) (Figure 1).

Water temperature Water temperatures ranged 17.8°-19.9°C (average 18.9°C) for the minimums, and 24.3°-27.1°C (average 26.3°C) for the maximums.

DISCUSSION

Among the biopesticides categorized by the US EPA including microbials, biochemicals and plant incorporated protectant (PIP), S-methoprene and its sibling compounds S-hydroprene and S-kinoprene are the only IGR biopesticides (US EPA 2021). These compounds have similar molecular structure and identical function to the natural juvenile hormones, doubled with their benign environmental and non-target profile (Henrick 2007, Su 2018). S-methoprene has very high bioactivity as indicated by low IE concentration at parts per billion (ppb) scale, low risk of resistance development and lack of cross-resistance to other larvicides. For example, field-collected mosquitoes that showed high levels of resistance to *Bacillus sphaericus* and pyriproxyfen, remained susceptible to S-methoprene. On the other hand, mosquitoes that have acquired high levels of resistance to S-methoprene through laboratory selection still showed high susceptibility to other commonly used pesticides except *B. sphaericus*, an obvious case of cross-resistance (Su et al. 2019a, b, 2021). Taking the advantages of advancement in formulation technologies, different products ranging from CS (5% and 20%), various granules (0.3% SBG and 4.25% P35), pellets (4.25%), briquets (8.62%) and extended residual briquets (2.1%) under teh trade name of Altosid® (Central Life Sciences 2022) that are customized for habitats and mosquito species have been developed and used in mosquito control operations since early 1970s. While slow-release formulations provide extended period of control and save labor cost in field operations, the sublethal exposure, one of the leading causes to resistance development (Su et al. 2021), seems unavoidable. While the resistance risk is low in S-methoprene, its actual status in the field populations of mosquitoes may have been overlooked after many years of applications.

The laboratory bioassay is critical to evaluate prototype or commercial products to prompt the subsequent field evaluations. In this paper, all actual, not projected, IE_{10}, IE_{50} and IE_{90} with their 95% CIs were obtained from dose-responses covering approximately 5-95% cumulative mortality. The significantly higher activities in *Ae. aegypti* and *An. hermsi* than in *Cx. quinquefasciatus* may imply higher effectiveness against the same species under field conditions. The species-dependent susceptibility to S-methoprene was also observed by others at various extents, where data showed generally higher susceptibility in floodwater mosquito species than others (Lowe et al. 1975, Ritchie et al. 2017, Su et al. 2019a, b). The species-dependent variations in susceptibility also exists in other commonly used mosquito larvicides, for instance, the species in *Stegomyia* group namely *Ae. aegypti* are much less susceptible to *B. sphaericus* (Su et al. 2019b). The formulated suspension showed the similar adult emergence inhibition as the technical grade S-methoprene, indicating the microencapsulating process did not appear to interfere with its insecticidal activity. In terms of stage-specific mortality across the dose-response range in laboratory bioassay, there seemed less mortality at “puparvae” as compared with that in pyriproxyfen (Su et al. 2020), because mortality mostly occurred at pupal stage, plus minors during larval stage at the higher concentrations and some mortality as incomplete emergence at the lower concentrations.

For ephemeral habitats or univoltine species, short-persistent formulations with adequate longevity are desired. A couple of CS formulations that have the similar product profiles and use patterns as the OmniPrene 20CS were made available decades ago such as Altosid liquid larvicides (A.L.L.) with 5% and 20% active ingredient. However, the labels of these products do not provide the efficacy longevity, rather advise to repeat the treatment as breeding sites become reinfested or when monitoring indicates an increase in adult populations (Central Life Sciences 2022). The field evaluations on the efficacy longevity of A.L.L. are quite meager (McCarry 1996, Webb et al. 2012). The new product OmniPrene 20CS is formulated with innovated proprietary microencapsulation technologies and provided 86.4-100%, 89.8-100% and 79.8-100% control against *Ae. aegypti*, *An. hermsi* and *Cx. quinquefasciatus*, respectively, over a 21-day evaluation period. This CS formulation, under the proprietary right and trademark protection, encapsulated the S-methoprene in a novel polymer shell suspended in water with a dispersant and wetting agent (Gimeno 1996, Dubey et al. 2009, Lam et al. 2010).

In mosquito species, late instar larvae are more susceptible than the early instar larvae to external JHAs such as S-methoprene and pyriproxyfen due to the significant decline of internal juvenile hormone III (JH-III) when completing larval growth and approaching pupation (Noguchi and Ohtaki 1974). Young larvae have low susceptibility to JHAs as their internal JH-III remains high to regulate larval growth. When treating a habitat with mixed larval instars at the label dose of a S-methoprene product, only old larvae that are approaching pupation will be mostly affected, ending up with dead pupae or incompletely emerged adults. The younger larvae however are considered being exposed to the sublethal dose of the active ingredient, a leading cause of resistance evolution. Hence, resistance monitoring for S-methoprene product is highly encouraged before and after applications. In contrast to direct kill larvicides, either conventional synthetic pesticides or ones with microbial origins, the unique mode of action of S-methoprene, i.e., allowing normal larvae growth and development, and only interrupting the pupal development and adult emergence, would allow the minimum impact to the trophic webs in aquatic habitats. However, this mode of action can mislead to a perception of low efficacy as normal-looking larvae do prevail after treatment, this scenario can be an issue for the public before the mode of action in S-methoprene and other JHAs is understood.

Currently, the CS formulations remain one of the most advanced formulation types for pesticide products worldwide. When CS formulations are diluted with water in the spray tank, a spontaneous suspension is formed, with particles in the size range of 0.1 to 20 μm. When sprayed, the dilute emulsion gives a uniform and accurate application of active ingredient onto the water surface, which is essential for effective control of target mosquito species. It is desirable to provide controlled release of S-methoprene as well as prevent degradation of the active ingredient by the innovated CS formulation technology. One can anticipate that the new product OmniPrene 20CS will soon be added to the toolbox that is at a historical all time low as we are facing the demand of more sustainable mosquito management.

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CO-OCCURRENCE OF MOSQUITO COMMUNITIES IN DISTURBED ENVIRONMENTS USING MARKOV RANDOM FIELDS (MRFs) IN ST. JOHNS COUNTY, FLORIDA

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ABSTRACT

The distribution of mosquito communities is predicted by complex micro- and macrohabitat systems. While macrohabitat variables are significant in modeling the distribution of individual mosquito species, the distribution of mosquito communities in disturbed urban and semi-urban environmental gradients was overlooked in most of the previous models. In our study, we used conditional Markov Random Fields (CRF) to evaluate spatial co-occurrence patterns between mosquito vectors of eastern equine encephalitis (EEEV) and west Nile virus (WNV) in a disturbed urban environment in Saint John's County, Florida. We aimed to 1) quantify the strength and direction of spatial unconditional and conditional correlations between mosquito assemblages in disturbed environments, and 2) evaluate whether the strength of correlations between mosquito assemblages is conditional on landscape or climate variables. We leveraged the longitudinal surveillance effort using Biogents sentinel traps (BGS) conducted by Anastasia Mosquito Control Districts in disturbed urban environments during 2017-2020. The distribution of high mosquito abundance, especially *Aeded albopictus*, *Ae. aegypti*, *Ae. vexans*, *Ae. taeniorhynchus*, *Culex nigripalpus*, *Cx. salinarius*, and *Cx. quinquefasciatus*, were conditionally correlated with other EEEV and WNV vector species in reduced woody and herbaceous wetlands and evergreen forests (-54.44%), and in urban developed landscapes (3.44%) during 2019 and 2020. Moreover, conditional correlations between mosquito species pairs were positively associated with increased total precipitation and in areas with high average minimum and maximum temperatures. Our results show that the micro- and macrohabitat characteristics demonstrated spatial effects on distribution and correlations between species pairs of EEEV and WNV mosquito vectors across disturbed environments. Our findings could be used to better understand the joint effects of drivers on mosquito diversity at a specific locality, interspecific interactions among mosquito assemblages, and how this diversity changes across environmental gradients.

Key Words: interspecies interaction, spatial distribution, conditional Markov Random Fields, host-seeking mosquito, community ecology.

INTRODUCTION

The transmission dynamics of mosquito-borne diseases are predicted by complex biophysical systems of host diversity, landscape, and interspecific correlations among disease vectors and host diversity (Pavlovskiĭ 1966). Available publications on disease ecology focus primarily on the biodiversity of vertebrate hosts and much less emphasis has been placed on a single vector species when considering arbovirus transmission dynamics (Keesing, et al. 2010; Kilpatrick, et al. 2006; Schmidt and Ostfeld 2001). In the meantime, mosquito vectors constitute broader multi-species assemblages that vary in composition, abundance, and vector competency to transmit pathogens, which can collectively impact pathogen transmission in a geographic area (Franklinos, et al. 2019; Johnson, et al. 2015; Lord 2009; McMillan, et al. 2020; Roche, et al. 2013). A key challenge in predicting local-scale species distributions and community composition is accounting for covariance between host diversity, host density, landscape, and biotic interactions. Available approaches have been focusing on exploring the relationships between the abundances of potential competitors in mosquito assemblages and vegetation gradients (Jansen and Oksanen 2013), while others have used pairwise probability calculations between individual species (Clark, et al. 2018; Veech 2013; Veech 2014), with some similarities across methods (Arita 2016). When considering landscape-scale co-occurrence, a particularly powerful approach, and yet unused for mosquitoes, is to first quantify dependencies between species pairs and then determine whether the strength of these dependencies is conditional on environmental variables (Clark, et al. 2018). This approach simultaneously considers micro- and macrohabitat factors that may be control the shape of species abundances, distributions, and community composition across space and time. The end goal of using such approaches is to better understand the joint effects of drivers on mosquito diversity at a specific locality, interspecific interactions among mosquito assemblages, and how this diversity changes over time across environmental gradients.

Here we focus on the use of mosquito control district data to examine the co-occurrence patterns of host-seeking female mosquito vectors of West Nile (WNV) and eastern equine encephalitis viruses (EEEV) in response to landscape-climate interactions in urban and peri-urban settings. Both WNV and EEEV pose a substantial risk to humans and livestock throughout the state of Florida. These viruses are maintained in the natural environment, primarily between avian hosts and mosquito vectors, although additional vertebrate hosts may be involved in each transmission cycle (Bingham AM, et al. 2014; Blitvich BJ 2008; Graham SP, et al. 2012). Several primary and bridge competent vectors are involved in transmission of these viruses throughout Florida, making monitoring and surveillance an important priority to inform management and control decisions (Lloyd, et al. 2018). Accordingly, mosquito control districts routinely trap and record mosquito counts identified to the species level and include metadata about the geographic location, the trap type, and the duration of the trapping effort (Lloyd, et al. 2018). Anastasia Mosquito Control District (AMCD), St. Johns County, FL, is one such district with a robust surveillance program. AMCD monitors mosquito species compositions and abundances on a weekly basis (January-December) using a network of trapping sites distributed across the county.

For this study, we leveraged longitudinal mosquito control district data from AMCD to quantify co-occurrence probabilities between species pairs in disturbed environments. We focused specifically on laboratory confirmed WNV and EEEV competent mosquito vectors to summarize potential co-distributions under different environmental conditions and to identify the potential for individual species abundances to inform predictions of vector occurrences in the absence of direct observations. We hypothesize that WNV and EEEV vectors have strong co-occurrence patterns with other mosquito species and that these are conditionally dependent on landscape factors. Alternatively, it may be that there is no conditional dependence and that landscape features are stronger predictors than interactions among mosquito community members in determining site level species abundances. Our objectives are to (1) evaluate the strength and direction of co-occurrence probability between mosquito assemblages, without environmental covariates, and (2) evaluate the conditional relationships between mosquito assemblages across environmental gradients.

MATERIALS AND METHODS

Sampling design and data collection

The BG trap surveillance program at AMCD started in 2014 but was newly prioritized in 2016 in response to the alarming emergence of Zika virus in the Americas (https://www.news4jax.com/news/2016/07/30/new-mosquito-trap-aids-in-fight-against-zika/). BG trapping is focalized within the City of St. Augustine, a highly residential and tourist hub spanning the Atlantic coast and an intercoastal waterway. The active surveillance season for BG traps is approximately 48 weeks of the year, lasting from early January to mid-December. Mosquitoes are collected by AMCD personnel using BG sentinel traps (Biogents; Moorefield, WV) paired with a 12 V battery, BG lure, and CO_2 attractant (dry ice) (KeHe Distributors Inc; St. Augustine, FL) once a week. Traps are deployed in the field for 18-24 hours and then collected mosquitoes were transported to the facility laboratory and identified to species level using taxonomic keys (Darsie and Ward 2005). The AMCD personnel identified, counted, and recorded all collected female mosquitoes from the 12 sites that were consistently sampled weekly throughout 2017-2020. Mosquito species that were listed in the database but had a zero average abundance for each of the four years were excluded from analyses. The latitude, longitude, and unique identifying numbers (UID) of all consistent trap locations (n=12) were imported into ArcMap v.10.8 (Esri; Redlands, California, USA) and 1km and 5km buffer radii were generated around each sampling locality (Fig. 1).

Environmental data

All land-use land-cover (LULC) data for years 2017-2020, released by the United States Geological Survey (USGS), with a spatial resolution of 256m were imported into ArcGIS 10.8. (https://www.sciencebase.gov/catalog/item/-5b96c2f9e4b0702d0e826f6d; compressed file name = CONUS_A1B_y20XX). The projected A1B scenarios for LULC dataset were selected for the current analysis, which represented the most optimistic scenario of greenhouse gas effect. The LULC raster were extracted for the study sites and clipped around the generated buffer radii. The LULC area percentages within each buffer radius were linked to the corresponding sampling site for further spatio-temporal analysis. A total of eleven landscape variables were identified within buffer radii and included in further analysis (Water, Developed, Mechanically Disturbed Private, Barren, Evergreen Forest, Grassland, Shrubland, Cropland, Hay/Pastureland,

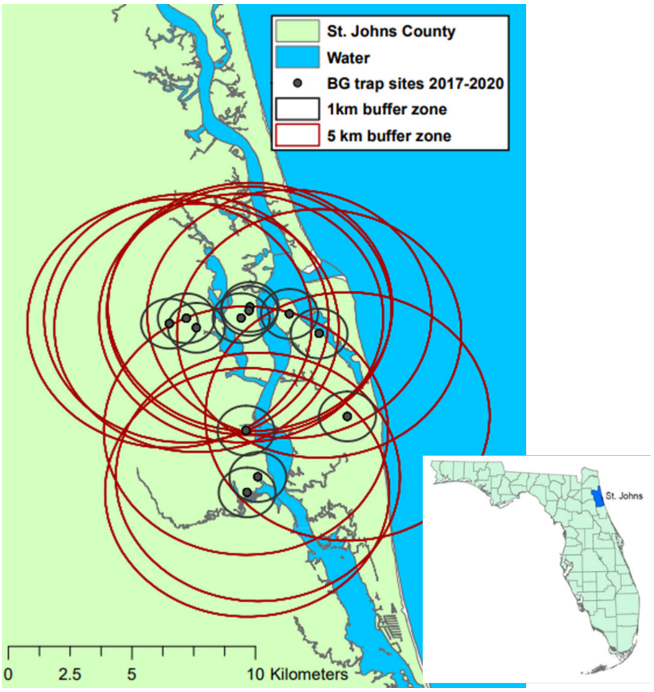

Figure 1. Map of the city with sampling sites (twelve consistent locations 2017-2020) with 1km and 5km buffer zones.

Herbaceous Wetland, Woody Wetland).

Climate variables at 800m spatial resolution (precipitation, minimum temperature, maximum temperature, mean temperature, minimum vapor pressure deficit, maximum vapor pressure deficit) were retrieved from *Parameter-elevation Regressions on Independent Slopes Model* (PRISM) Historical Data explorer (https://prism.oregonstate.edu/explorer/) using the 'dismo' package in R. Daily climate values 2017-2020 were then compiled into annual averages.

Statistical analyses

Average annual mosquito abundances of each species per site (female mosquitoes/number of active trap nights/surveillance season) were calculated to first evaluate the spatial interspecific interactions between mosquito communities using Markov Random Fields (MRF) analyses. Next, the average annual mosquito abundances per site were spatially joined to landscape and climate variables to next evaluate the strength of interspecific interactions across different landscapes using Conditional Random Fields (CRF) analyses. The analyses were conducted using MRF which provides spline functions for approximating interaction parameters of species in undirected graphical networks (Clark, et al. 2018). Meanwhile, CRF offer greater flexibility than previous co-occurrence methods because this approach estimates correlations among nodes (i.e., species) and then calculates whether a variable pair is conditionally dependent on a third variable (i.e., environment), providing information about the strength level and direction of correlations. To evaluate the annual co-occurrence probabilities between mosquito assemblages, four analyses were conducted separately for four time series during 2017-2020.

To prepare data for analysis, we used Sallam et al. (2023). Average mosquito abundances per trap day were rounded to an integer value to serve as nodes (species). Percent landscape and average bioclimatic variables were added to their corresponding mosquito collections sites within buffer radii to serve as the covariates in the model. To calculate CRF using abundance data, pairwise linear regressions across all combinations of species and environmental variables were carried out by using an optimized regularization multiplier for variable selection and to reduce overfitting. Afterwards, predicted and observed values for species pairs combinations were used in model validation (Clark, et al. 2018). The spatial spline was used by including geographic coordinates of trap locations to account for residual spatial autocorrelation (Banerjee, et al. 2004). A 500 bootstrap analyses replicates using Poisson method along with all data points, with random replacement of data points in each replicate, were conducted to evaluate uncertainty in correlation estimates (Clark, et al. 2018). All key regression coefficients of each species, each year, and all years in combination with a threshold value of >0.01, only, were output to a single *.csv table showing the relative importance and mean coefficient values of each variable (Table 1-3, Table S1). Two separate models, unconditional and conditional, were run within each of the 1 km and 5 km buffer distances.

RESULTS

A total of 2,125 mosquitoes represented 15 species during 2017 and 2018, and 16 species in 2019 and 2020 were sampled during January - Mid-October. Initial exploration of mosquito diversity and abundances by genera across the eight trap sites indicated variability across the twelve consistent trap locations. The species composition and abundance at these sites were dominated by the high density of *Aedes* species, especially *Ae. aegypti* and *Ae. albopictus*, during 2017 and 2018, and by the high density of *Culex* species, mainly *Cx. quinquefasciatus*, *Cx. nigripalpus*, and *Cx. salinarius*, during 2019 and 2020 (Figure 2). Additionally, landscape structure has witnessed changes during the study period demonstrated by the change in area percentages of different landscape types, especially during 2019 and 2020. For example, in 2020, the area percentage of developed landscapes significantly

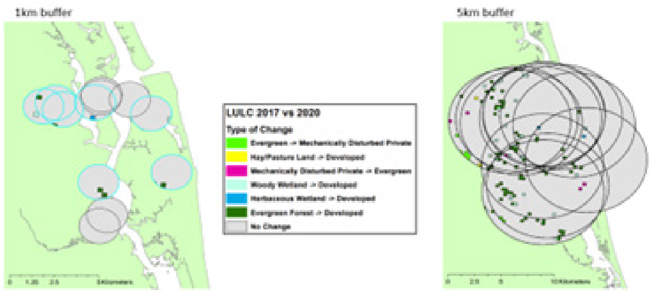

Figure 2. Spatial changes in LULC from 2017-2020 within 1km and 5km buffer radii (refer to scale bar in each image). Left: The blue highlighted 1km buffer radii show areas with LULC change only. Right: All 5km buffer radii zones show areas with LULC change.

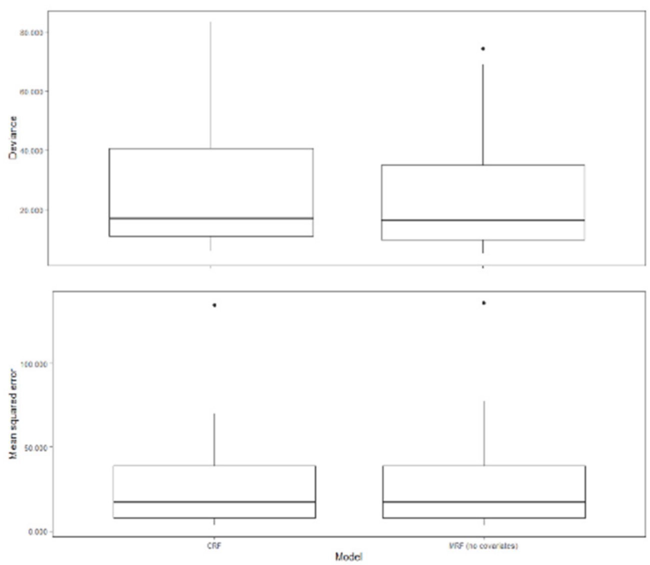

Figure 3. Box plots of CRF analyses with (left) and MRF analysis without covariates (right) show MSE and deviance within 5 km.

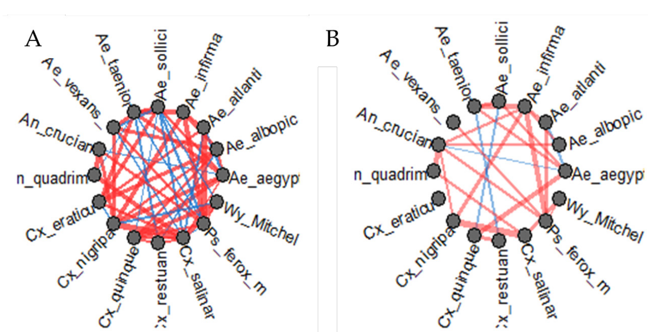

Figure 4. System network of co-occurrence probabilities between mosquito assemblages showing model without covariates A) and model with covariates within 1km buffer radii (B). The red and blue lines show positive and negative correlations, respectively. The thickness of the line demonstrates the strength of the correlation.

increased (3.44%) in a total of eight sampling sites within 1km buffer radii. Whereas area percentage of woody and herbaceous wetland and evergreen forest drastically reduced (-54.44%) in the same sites within 1km buffer radii (Figure 2).

Statistical Analyses

Box plots for bootstrapped models with no covariates (MRF) and with covariates (CRF) measured within 1 km and 5 km buffer distances indicated that the inclusion of landscape and bioclimatic variables did not significantly improve the overall model performance when evaluated by deviance (DV) and mean squared error (MSE) values (Figure 3 & S1 Figure). The insignificant differences in DV and MSE values, especially within 5 km, reflect that the change in number and strength of correlations between species pairs, as indicated by relative importance values, did not change the overall spatial co-occurrence correlations between mosquito species pairs.

The network plots generated for CRF models without (Figure 4A, S2A) and with environmental variables (Figure 4B, S2B) showed that the number and direction of correlations between individual species pairs were slightly impacted by the environmental variables. Additionally, the strength of correlations between individual species pairs varied with the inclusion of an environmental variable (Figure 4B, S2B). The reduced number of species pairs in Plots 4B and S2B compared to Plots 4A and S2A indicated that several species pairs were highly correlated when no environmental variables were included in the model, but that these correlations reduced to zero when environmental variables were added. Also, it reflects that individual mosquito species are correlated with environmental variables, especially within 1km buffer radii (Figure S2).

Overall, the regression coefficients in tables 1-3 and S3 indicated that a greater number of vector species were unconditionally correlated on another mosquito vector species compared to the environment; however, some species did exhibit correlations with landscape and climate variables and several species pairs were no longer correlated with one another when environmental variables were added. The number and strength of unconditional correlations between species pairs within 5km (n=18) and 1km (n=3) were significantly different which was indicated by the relative importance values (Table 1, S3). For the 5km buffer radii models, the log abundances for 13 WNV and EEEV vector species were correlated with another mosquito species and not on environmental variables within 5km buffer distances (Table 1). *Aedes albopictus* is the only species that demonstrated conditional dependence on minimum temperature values (relative importance

= 0.031) within 5km buffer radii, especially during 2017. Results from 1km models indicated that three species pairs showed unconditional correlations with other mosquito vector species and four species pairs showed conditional correlations with other vector species at areas with increased minimum and maximum temperature values (Table 3). Additionally, the increased log abundances of two vector species, *Ae. albopictus* and *Cx. nigripalpus*, were conditionally correlated with environmental variables (minimum and maximum temperature values), especially during 2018 and 2019, as indicated by relative importance and correlation coefficients.

DISCUSSION

The response of species composition and abundance of mosquito disease vectors to landscape changes has been evaluated in urban and peri-urban areas in SJC to prioritize areas of vector control efforts, especially during outbreaks and after natural disasters such as hurricanes. Landscape changes due to anthropogenic activities have largely contributed to either creating new habitats or reducing habitats for mosquito species (Keesing, et al. 2010; Schmidt and Ostfeld 2001). This is also associated with changes in the microclimate of these habitats which increase/ decrease the abundance, diversity, and distribution of mosquito species. For the first time, we investigated abundances of WNV and EEEV mosquito vector species in a changing environment using a community ecology approach that quantified dependence on landscape and climate variables, as well as other mosquito species, and then determined if and how the strength of dependence between species pairs varied across environments. The result is a novel view of mosquito vector co-occurrence in the context of highlighting the potential to use species co-occurrences within mosquito communities as indicators of vector abundances in the absence of direct observations in disturbed environments.

The diversity of host-seeking mosquito vectors and their spatial and temporal co-occurrences in changing environments have been highlighted in previous studies to play an important role in introducing mosquito-borne diseases to new areas (Franklinos, et al. 2019; Keesing, et al. 2010; Lord 2009; Roche, et al. 2013; Schmidt and Ostfeld 2001). Based on previous empirical observations from these studies (Estep, et al. 2013; Sallam, et al. 2016a; Sallam, et al. 2016b), we expected to find that log abundance of vector species would be dependent on the landscape and climate variables. We also anticipated a significant change in mosquito diversity between years due to habitat loss or gain. Surprisingly, a total of ten of thirteen 13 WNV and EEEV vector species showed unconditional dependence on other vector species but not on environmental variables within 1km and 5km buffer radii. This may indicate that these vectors are typically broad-habitat generalists and reflect the reduced effect of environmental factors on these studied vector species, which can present challenges when investigating occurrence patterns using environmental variables alone in changing environments. For example, the increased number of correlations between *Ae. taeniorhynchus* (saltmarsh mosquito) and *Ae. infirmatus* (floodwater mosquito) reflect the increased expansion range of the former species in response to development projects that result in changing environments especially within coastal areas of St. Johns County. This has been reported in many coastal regions in the south and central Florida, especially in brackish water habitats around mangrove areas (Qualls, et al. 2021). However, some previous studies indicated *Ae. taeniorhynchus* is a habitat specialist species in areas with low mean diurnal temperature ranges, which almost certainly reflects its coastal affinities (Fehring 1986; Qualls, et al. 2021; Sallam, et al. 2022; Shaman, et al. 2002), where residual heating or cooling from ocean temperatures reduces onshore fluctuations in temperatures. Our results also showed that three mosquito vectors species out of 13 WNV and EEEV mosquito vectors were found to strongly depend on two climate variables (maximum temperature and annual precipitation) within 1km buffer radii only. Our results indicated that the increase of maximum temperature values positively affected the log abundance of *Ae. albopictus* and *Cx. nigripalpus*, which was consistent with previous studies (Sallam, et al. 2016b; Sallam, et al. 2017). Equivocally, the log abundances of *Ae. taeniorhynchus* drastically decreased with heavy precipitation due to hurricane Irma during 2017 (Qualls, et al. 2021).

We observed a significant variation in the number and strength of unconditional dependences measured within 1km compared to 5km buffer distances of trap locations. This was confirmed by the smaller number of unconditional correlations between species pairs within 1 km (n=3) compared with those within 5 km (n=18). There was a strong co-occurrence probability between *Cx. restuans* versus *Cx. quinquefasciatus* and *Cx. quiqnuefasciatus* versus *Ae. aegypti*, but these three were the only WNV vectors that showed strong correlations within 5km buffer radius. This observation is consistent with similar studies in other regions in Florida (Sallam, et al. 2022). This also may reflect general occurrence across landscape types, but more specific, shared meso- or micro-scale habitat preferences that were not measured here. Moreover, this could reflect the potential for unmeasured covariance between spatial and temporal niche dynamics, especially given these taxa are known to be tied to the dynamics of wet season timing in north and central Florida (Ferreira-de-Freitas, et al. 2020; Sallam, et al. 2016b). Increasing our buffer radius from 1km to 5km resulted in capturing greater variability in climate and landscape conditions, which may be only marginally variable across smaller geographic areas such as the City of St. Augustine in SJC. Considerations of scale in the use of such approaches are particularly important, especially given the complexities of interpreting co-occurrence (or co-abundance) in relation to (here unmeasured) microhabitat drivers. Although landscape changes did not affect species composition, these changes had a minor spatio-temporal effect on the abundance of mosquito species during 2019 and 2020 compared to 2017 and 2018. That was demonstrated by the increased abundance of *Culex* mosquitoes during the last two years of sampling in eight sampling locations compared to *Aedes* species which were abundant during the first two years of sampling in the same sampling locations. Also, any minor landscape changes, especially developed, woody and herbaceous wetland, potentially brought WNV and EEEV mosquito vectors together within their flight ranges, which may have an impact on transmission dynamics especially during outbreaks. For example, the positive co-occurrence probabilities between *Cx. nigripalpus* versus *Cx. salinarius*, *An. quadrimaculatus* versus *An. crucians*, *Ae. albopictus* versus *Ae. vexans*, and *Ae. albopictus* versus *Cx. quinquefasciatus* may reflect intraspecies associations in their respective breeding sites. Also, the reduction in woody and herbaceous wetland and evergreen forest during 2019 and 2020 may have had an impact on diversity and abundance of reservoirs due to the habitat loss and range compressions of bird reservoir hosts, unmeasured in the current study. Additionally, the habitat loss during these years may have also brought multiple mosquito vectors into areas with altered mesohabitats. The dynamics of diversity and abundances in reservoir hosts and mosquito communities have been highlighted in previous studies to impact the WNV and EEEV seroconversions and spillovers. In SJC, both WNV and EEEV positive seroconversions were only reported in 2019 and 2020, whereas in 2017 and 2018, only EEEV was only reported in both years.

Interpreting co-occurrence results in the context of the underlying mechanism of interaction/competition between species pairs is very challenging, especially since our analyses mainly focused on solely host-seeking female mosquitoes. The lack of immature stage data from breeding/development habitats was a major limiting factor in evaluating interspecific interactions between species pairs. However, our approach can provide a basis for more detailed studies that include breeding habitats attempting to demonstrate biotic interactions or reflect differential micro-scale habitats in breeding habitats. The negative co-occurrence probabilities between species pairs in our results may be due to microscale patterning rather than direct competition, such as the negative co-occurrence probability between *Cx. quinquefasciatus* and *Ae. sollicitans.* The former is a generalist in open permanent and semipermanent pools, lakes, ponds, lakes, and swamps (Linley, et al. 1986), and the latter prefers saltmarshes (Hopperstad and Reiskind 2016; Hribar, et al. 2004; Reiskind and Lounibos 2009).

Although the longitudinal adult mosquito surveillance efforts in SJC was robust, additional surveillance techniques are needed to include other mosquito life stages. For example, the spatial conditional dependencies between adult species pairs, concluded in our results, need further studies to highlight the seasonality of these dependencies in mosquito immature habitats. Additionally, because our study focused on host seeking female mosquitoes in disturbed habitats, further investigation into the contribution of mosquito phenology is warranted. The purpose of this study was not to dissect the underlying dynamic of community abundances across disturbed habitats; however, our results shed the light on the co-occurrence between mosquito vectors in the context of understanding transmission risk in disturbed environments.

CONCLUSIONS

Spatial distribution of abundance and diversity of mosquito vector species have been highlighted in previous studies as an indicator of EEEV and WNV spillover events in Florida. However, most of these studies overlooked the impact of micro- and macrohabitat variables on diversity and abundance of mosquito communities. Specifically, the strength and direction of correlations between mosquito vector species pairs across landscape have not been highlighted in the context of community ecology. We leveraged the surveillance effort data from AMCD to quantify strength and direction of correlations between EEEV and WNV mosquito vectors in disturbed environment. The majority of mosquito vectors of EEEV and WNV were positively correlated with other vector and non-vector mosquito species, without environmental variables. Correlations between few species pairs were affected by adding the environmental variables, especially within 1km buffer radii. For example, the spatial distribution of seven mosquito species, *Ae. albopictus*, *Ae. aegypti*, *Ae. vexans*, *Ae. taeniorhynchus*, *Cx. nigripalpus*, *Cx. salinarius*, and *Cx. quinquefasciatus*, were conditionally correlated with other EEEV and WNV vector species. These conditional correlations may reflect the significant effect of reduced woody and herbaceous wetlands and evergreen forests (-54.44%), and expansion of urban developed landscapes (3.44%) during 2019 and 2020. Also, three climate variables, increased total precipitation in areas with high average minimum and maximum temperatures, were associated with the conditional correlations between these species. Generally, the habitat generalist mosquito vector species demonstrated low number of conditional dependences on environmental variables. Our approach could be used to leverage species co-occurrences under scenarios where environmental variables are not informative.

Supplementary Materials: Figure S1: Lambda plot of bootstrapped models with (CRF) and without covariates (MRF) showing the MSE and deviance values within 1km; Figure S2: System network of co-occurrence probabilities between mosquito assemblages showing model without covariates A) and model with covariates within 1km buffer radii (B); Table S1: Unconditional dependence between species pairs of vectors within 1Km buffer radii.

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Table 1. Unconditional correlations between vector species pairs within 5km buffer distances.

Species name	species variable name	Relative importance	10% Quantile	Mean coef.	90% Quantile
Ae. albopictus[3] (Turell et al. 1994 & 2001)	*Cx. quinquefasciatus*[1]	0.882	0.000	0.061	0.061
	Ae. infirmatus[2]	0.087	0.019	0.019	0.019
Ae. atlanticus[1]	*Ps. ferox*	0.997	0.180	0.180	0.180
Ae. infirmatus[2] (Wellings et al. 1972, Vaidyanathan et al. 1997)	*Cx. nigripalpus*[1]	0.113	0.118	0.118	0.118
Ae. sollicitans[2] (Hayes, 1981, Hubálek et al. 2014)	*Ae. taeniorhynchus*[1]	0.663	0.131	0.131	0.131
	Cx. quinquefasciatus	0.170	-0.066	-0.066	-0.066
	Ae. aegypti[1] (Turell et al. 2001)	0.167	0.066	0.066	0.066
Ae. taeniorhynchus[1] (Turell et al. 2001)	*Ae. infirmatus*[2]	0.758	0.237	0.237	0.237
Ae. vexans[3]	*Ps. ferox*	1	0.025	0.025	0.025
An. crucians[1] (Gubler et al. 2007, Mackay 2007)	*Ae. aegypti*[1]	0.41977058	-0.197	-0.197	-0.197
	Ae. albopictus[1]	0.04082455	0.061	0.061	0.061
	Ps. ferox	0.01233099	0.034	0.034	0.034
An. quadrimaculatus[3] (Molaei et al. 2008, Vaidyanathan et al. 1997)	*An. crucians*[1]	0.95708216	0.219	0.219	0.219
	Cx. salinarius[3]	0.04291777	0.046	0.046	0.046
Cx. nigripalpus[1] (Sardelis et al. 2001)	*Cx. salinarius*[3]	0.93466611	0.452	0.452	0.452
Cx. quinquefasciatus[1] (Vaidyanathan et al. 1997)	*Ae. aegypti*[1]	0.91491990	0.324	0.324	0.324
Cx. restuans[1]	*Cx. quinquefasciatus*[1]	0.98545842	0.073	0.073	0.073
Cx. salinarius[3] (Molaei et al. 2006, Anderson et al. 2012)	*Wy. mitchellae*	0.07266669	0.127	0.127	0.127

Table 2. Conditional correlations between vector species and environmental variables within 5km buffer radii.

environmental variable	Species name	Buffer radius (km)	Relative importance	10% Quantile	Mean coef.	90% Quantile
Tmax during 2018	*Ae. albopictus*	1	1	1.375	1.375	1.375
	Cx. nigripalpus	1	0.048	0.233	0.233	0.233
Tmax during 2019	*Cx. nigripalpus*	1	0.952	1.037	1.037	1.037
Tmin during 2017	*Ae. albopictus*	5	0.031	0.012	0.012	0.012
Precip during 2017	*Ae. taeniorhynchus*	1	0.504	-0.001	-0.001	-0.001

Table 3. Conditional correlations between vector species pairs within 1km buffer radii.

Environmental variable	Species name	Species variable name	Relative importance	10% Quantile	Mean coef.	90% Quantile
Tmax during 2017	*Ae. aegypti*	*Ae. albopictus*	0.061	0.001	0.001	0.001
		Ae. taeniorhynchus	0.477	0.001	0.001	0.001
	Cx. salinarius	*Cx. nigripalpus*	0.020	0.001	0.001	0.001
Tmin during 2017	*Cx. salinarius*	*Cx. nigripalpus*	0.833	0.008	0.008	0.008

Appendix A

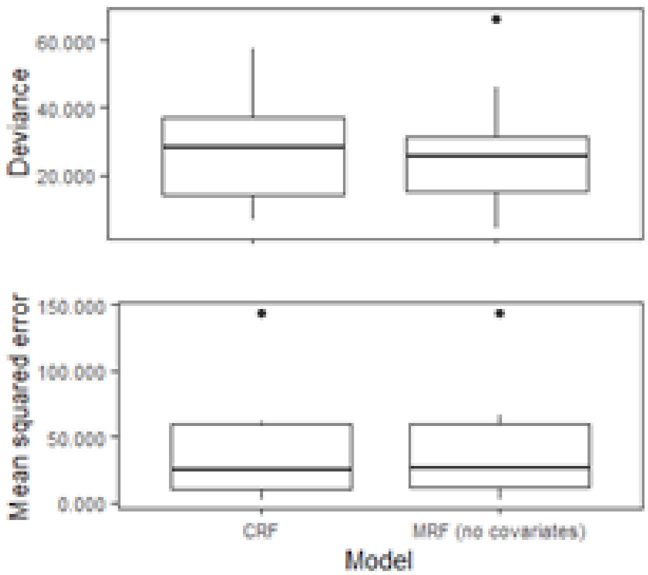

Figure S1. Lambda plot of bootstrapped models with (CRF) and without covariates (MRF) showing the MSE and deviance values within 1km.

Appendix B

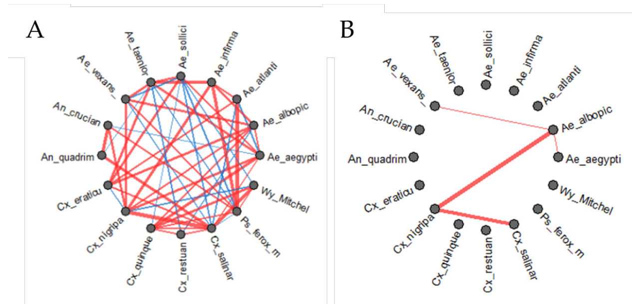

Figure S2. System network of co-occurrence probabilities between mosquito assemblages showing model without covariates A) and model with covariates within 1km buffer radii (B). Red and blue lines show the positive and negative correlations, respectively. The thickness of the line demonstrates the strength of the correlation.

Appendix C

Table S1. Unconditional dependence between species pairs of vectors within 1Km buffer radii.

Species name	species variable name	Relative importance	10% Quantile	Mean coef.	90% Quantile
Ae. aegypti[1]	*Ae. taeniorhynchus*[1]	0.033	0.001	0.001	0.001
Ae. vexans[3]	*Ae. albopictus*[1]	0.996	0.001	0.001	0.001
Cx. salinarius[1]	*Cx. nigripalpus*[1]	0.146	0.48	0.48	0.48

FIELD EVALUATION OF LURES AS CANDIDATE ATTRACTANTS FOR COASTAL *CULICOIDES* IN FLORIDA

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ABSTRACT

Biting midges (Diptera: Ceratopogonidae: *Culicoides*) along the coast of Florida are a severe biting nuisance that can impede outdoor activities. Methods currently available for biting midge control are limited due to environmentally sensitive larval habitats and the lack of adulticide techniques available for area-wide population suppression.

Homeowners that live along the coast can protect their homes with the use of fine- meshed window screens and fans, coarse low volume adulticide applications, and adult removal via commercial mosquito traps. There are known attractants that have been used to enhance trap captures for adult mosquitoes, and it is logical to test these attractants against closely related adult hematophagous Diptera such as *Culicoides*. This study compared four attractant lures, octenol, BG lure, R-octenol, and USDA red blend, known to be attractive to mosquitoes, and tested them against *C. furens* and *C. mississippiensis* as an addition to increase trap capture. Although all attractants increased trap capture above the control, results from this study could not identify a lure that was significantly more attractive to *Culicoides* species captured in Cedar Key, Florida. Further studies evaluating species specific trap types as well as attractants are needed to develop an effective *Culicoides* adult trapping control system as part of an integrated biting insect control program.

Key Words: Biting midges, *Culicoides*, *C. furens*, *C. mississippiensis*, attractants

INTRODUCTION

Florida welcomed approximately 33 million visitors each quarter of 2022, an increase of over 15.3 percent more than in 2021 (visitflorida.com). As one of the top vacation destinations in the world, the state of Florida relies on income generated from coastal tourism to help fund the state's budget with residents and visitors expecting quality recreation and a comfortable outdoor environment. Florida's development history is directly linked to success in controlling mosquitoes (Diptera: Culicidae), abating the diseases they carry, and reducing the nuisance. These control measures can, to a lesser extent, reduce biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) but control after treatment is relatively short lived (Meloni et al 2018). The primary nuisance coastal biting midge species in Florida are *Culicoides furens*, *Culicoides barbosai* and *Culicoides mississippiensis* (Kettle 1968). These coastal *Culicoides* species are members of the order Diptera, one of the largest orders within the class Insecta. They are one of four haematophagous genera within the Ceratopogonidae family and are considered to have medical and veterinary importance world-wide due to their ability to transmit pathogens that cause disease in livestock, and their biting action (Kettle 1965, Linley et al. 1983). Methods currently available for biting midge control can be divided into 4 general categories: mechanical, chemical, biological, and genetic (Harrup et al 2016). Biting midges that are found along coastal areas in Florida use environmentally sensitive habitats for larval development that further restrict available control measures to adult exclusion, adulticide, and adult removal. Homeowners that live along the coast can protect their homes with the use of fine-meshed window screens and fans preventing biting midges from accessing the inside of the home. To reduce biting pressure around the home, coarse spray adulticide applications on foliage have proven to be successful with up to 85% reduction of adult biting midges (Standfast et al 2004 and Lloyd et al. 2021). These type of applications require maximum label rate applications every 4-6 weeks to reduce biting pressure. Commercially available adult mosquito traps are commonly used for mosquito surveillance to monitor mosquito population abundance (Li et al. 2016, Bazin and Williams 2018, Wilke et al. 2019). However, mosquito traps can also reduce mosquito populations (Kline 2006) acting as both a surveillance and control tool. Some of these mosquito traps also report large numbers of biting midges in the capture nets (Cilek et al 2003 and Lloyd et al. 2008). This observation suggests that mosquito traps should be considered as an additional tool for the homeowner to use as a strategy to help alleviate biting midge pressure around their home. Commercial mosquito traps rely on long and short range attractants to lure in and capture host seeking biting flies with many of these attractants being specific to species and /or genera.

Host-seeking biting midges are attracted by a multitude of long and short range cues such as visual, carbon dioxide (CO2), heat, and host hormone emanations (kairomones). As in most Diptera, biting midges use long range cues such as visual and light stimuli to initiate host location. Bishop et al. (2008) reported *Culicoides brevitarsis* Kieffer were attracted to two-dimensional cattle-shapes that were illuminated. As with live cattle, *C. brevitarsis* displayed strong preference for the back positions of the two- dimensional cow shapes suggesting that at least part of the attraction of *C. brevitarsis* was visual differentiation at the interface of the cow shape's back, and the background.

Holbrook and Bobian (1989) found that traps with an incandescent light source and a fan (with or without dry ice) increase catches of female *Culicoides variipennis* (Colquillett). Venter and Hermanides (2006) reported an increase in *Culicoides imicola* Kieffer captured in Africa using traps equipped with black light versus traps equipped with white light. To improve trap efficiency, light emitting diodes have been used as a visual attractant to enhance biting midge trap collections (Bishop et al. 2004, 2006).

Carbon dioxide detection by host seeking biting midges has been thoroughly studied and well documented as an effective attractant for *Culicoides* (Nelson 1965, Defoliart and Morris 1967, Takken and Kline 1989, Kline et al. 1990, Weiser-Schimpf et al. 1991, Ritchie et al. 1994, Mullens and Gerry 1998, Liu et al. 2009).

After following long-range cues to get closer to a potential host, there are multiple short-range cues such as heat, kairomones and synthetic chemical stimuli that may be utilized by the biting midge to target the host. Kline and Lemire (1995) were able to significantly increase CDC trap collection size with *Culicoides furens* Poey by the addition of heat. The authors reported that if efficiently delivered, heat could increase the effectiveness of barrier treatments targeting hematophagous insects. Kairomones are most likely to be the prevalent short-range cues used for host location. Previous studies have evaluated the effectiveness of traps baited with animals (Koch and Axtell 1979) and humans (Kitaoka et al. 2005). The addition of human and animal baits increase biting midge trap numbers; however, human and animal baits have been limited to the purposes of research and are not suitable for biting midge management.

Fortunately, synthetic attractants have been developed to mimic natural biological host-emitted cues and have moved to the front of attractant development for management of pestiferous insects. One of the most common attractants used for biting fly trap attractants is 1-Octen-3-ol (octenol), a component of ox's breath (Vale and Hall 1985). Octenol is widely used as an attractant for many hematophagous insects (Vale and Hall 1985, Anderson 1989; French and Kline 1989, Takken and Kline 1989, Kline et al. 1990, 1991) and has been evaluated against multiple *Culicoides* species and is generally considered to be effective (Takken and Kline 1989, Kline et al. 1994, Ritchie et al. 1994, Blackwell et al. 1996, Liu et al. 2009).

Octenol lure blends have been evaluated and proven to be effective but can be species specific depending on the component that is blended with octenol (Blackwell et al. 1996). For example, when octenol is combined with 4-methlyphenol (phenol), there is no significant difference in the number of insects captured in traps of four *Culicoides* species. However, when carbon dioxide (CO2) is added to the octenol-phenol mixture, two *Culicoides* species captured in trap collections are significantly increased (Cilek and Kline 2002).

With the exception of octenol, at the time of this study, there were no commercial lures specific for attracting host seeking *C. furens* to trapping devices. There are known attractants that have been used to enhance trap captures for adult mosquitoes, and it is logical to test these attractants against closely related adult hematophagous Diptera such as *C. furens.* The goal of this study is to compare four attractant lures, octenol, BG lure, R-octenol, and USDA red blend (Bernier et al. 2002, 2003), known to be attractive to mosquitoes, and test them against *C. furens* and *C. mississippiensis* as an addition to increase trap capture.

MATERIALS AND METHODS

Two tests were conducted between February and August 2009. Five MM-Liberty Plus® traps (Liberty Plus) (Wood Stream Corporation, Lititz, PA) were used to disperse the attractants in this study. Two commercially available attractants (BG Lure, Biogents AG, Regensburg, Germany; octenol (Wood Stream Corporation, Lititz, PA) and two experimental attractants (R-octenol, Bedoukian

Research, Danbury, CT); and USDA Red Blend (Kline et al. 2012) were assigned to a Liberty Plus and evaluated for attractiveness against field populations of *C. furens*. Five treatments were set in a 5 X 5 Latin square design, including the control (no attractant). All treatments were randomly assigned and rotated until all five treatments were evaluated at each location within the study site.

Study Sites

The first study was conducted from February through March 2009; *C. mississippiensis* is the dominant species captured in the study site during this time period. This study site consisted of a residential neighborhood (Rye Key) in Cedar Key, Florida. Rye Key is a 5.91 ha island surrounded by the Gulf of Mexico and has extensive marsh inlets; it is located at the Northeast tip of Cedar Key (Fig 1). This site was chosen because it consistently produced high numbers of *Culicoides* during a previous study conducted over one year (Lloyd et al. 2008). This site has limited access and is protected by an electronic gate reducing the chance of vandalism or theft of the equipment. The surrounding flora located at each site was similar (Fig 2 A, B). The larval biting midge habitat contained predominately Rush (*Juncus* spp.) and Cordgrass (*Spartina* spp).

Figure 1. Aerial view of Rye Key, a residential neighborhood in Cedar Key, Florida, in which the MM-Liberty Plus® traps were placed (trap treatment site 1).

Figure 2. A) Example of flora around one home on Rye Key. B) Example of flora around second home in a residential neighborhood (Rye Key) in Cedar Key, Florida. Pictures depict the similarity in treatment sites.

The second study was conducted from May through August 2009; *C. furens* is the major species during this time period. This study site consisted of a dense, forested habitat in the federally protected Lower Suwannee National Wildlife Refuge (LSNWR) located 22.5 km north of Cedar Key, Florida. This trapping site is on the Gulf of Mexico with extensive marsh inlets surrounding the south side of the trapping site. This site was chosen due to the high numbers of *Culicoides* it consistently produces (LSNWR wildlife ranger, personal communication) and access is limited by a locked gate reducing the chance of vandalism or theft of the equipment.

***Culicoides* Trap**

The Liberty Plus is a propane powered, counter-flow geometry trap (Fig 3). This trap is designed to mimic a vertebrate host through the combustion of propane to generate heat, moisture, and CO2 to attract biting insects (Kline et al. 2011). The trap produces approximately 550 ml/min of combusted CO2. This trap utilizes a thermoelectric module to capture some of the combusted heat to produce electricity that powers the suction fans. Propane is supplied by a standard 9.0 kg commercial propane tank. The average surface temperature of this trap is 37.1 °C and the plume temperature ranges from 33.3 to 40.6 °C. The Liberty Plus is cordless, has a push-button start, and lights that indicate when the machine is operating and if service is needed.

Figure 3. Mosquito Magnet Liberty Plus® trap used to evaluate four attractants in the capture of *Culicoides* on Rye Key in Cedar Key, Florida and Lower Suwannee National Wildlife Refuge. The Liberty Plus® is a propane powered, counter-flow geometry trap. This trap is designed to generate heat, moisture, and CO2 through the combustion of propane.

Attractants

One hundred fifty milliliters of Red Blend attractant was poured into a 250-ml glass Wheaton bottle, and daily change in Red Blend volume used during each repetition was recorded (Table 1). Originally developed as a mosquito attractant, Red Blend attractant is a composition of 1-octen-3-ol, 1-hexan-3-ol, and acetone (Kline et al. 2012). Red Blend was delivered via a single wick protruding from the center of a screw cap lid approximately 1 cm above the rim of the cap, referred to as "wick out" by Kline et al. (1991). The BG Lure, octenol, and R-octenol were used per manufacturer's recommendations. The Red Blend lure remained on the trap during each trial. All lures were secured to the attractant delivery system of the Liberty Plus trap and replaced with new lures for each trial (Fig 4, A-D).

Experimental Protocol

Five traps were randomly placed at least 50 meters apart and were rotated to the next location after each trap collection to minimize local hot spot effects. A 5 X 5 Latin square experimental design was used to ensure all treatments were consecutively rotated through the trapping locations resulting in five consecutive 24-h collection periods. Each five-day test in Study 1 was repeated three times. In Study 2, the five-day test was repeated four times.

Figure 4. Method for mounting four attractants on a MM-Liberty Plus® trap at trapping sites on Rye Key in Cedar Key, FL and the Lower Suwannee National Wildlife Refuge, from February-August 2009: A) BG Lure. B) Red Blend. C) Octenol and R-octenol. D) Close-up of attraction tube with octenol cartridge extended from tube for viewing purposes. Method for delivery of octenol and R-octenol lures was the same.

Upon returning to the laboratory, all field collections were immediately placed into a temperature controlled cold room to anesthetize the insects. Once the insects were immobile, they were placed into a 237 ml paper food cartons, labeled, and stored in a -20 °C freezer until processed. After removal from the freezer, the *Culicoides* were separated from the non-target insects using a 16-mesh copper screen, wire diameter 0.28 cm. If the *Culicoides* capture sample weighed over 0.025 grams (>500 flies), 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 *Culicoides* capture sample weighed less than 0.025 grams (< 500 flies), the entire collection

Table 1. Daily ranges of Red Blend volume used during each repetition, for all seven repetitions, from February to August 2009.

Trapping Day	Red Blend Volume Used (ml)	Volume Remaining (ml)
1	0	150
2	1-49	101-149
3	1-30	71-100
4	1-20	51-70
5	1-9	41-50

Table 2. Total number of Culicoides species caught in Cedar Key, FL and the Lower Suwannee National Wildlife Refuge from February to August 2009 using five MM-Liberty Plus® traps baited with four lures and a blank control.

Species	LSNWR	Cedar Key	No. of *Culicoides* spp. captured
C. furens	819,062	0	819,062
C. mississippiensis	1,356	604,762	606,118
C. insignis	0	474	474
C. melleus	1176	0	1176
Total	820,238	606,592	1,426,830

Table 3. Mean ± SE collection of *Culicoides furens* and *Culicoides mississippiensis* captured per MM-Liberty Plus® trap, separated by treatment, placed in the Cedar Key neighborhood on Cedar Key, FL (Study 1) from February to March 2009 and the Lower Suwannee National Wildlife Refuge (Study 2) from May to August 2009.

Attractant	*n*	*Culicoides furens* [a]	Attractant	*n*	*Culicoides mississippiensis* [b]
Octenol	20	10,594 ± 1,508 A	BG Lure	15	10,009 ± 1,809 A
BG Lure	20	8,529 ± 1,681 A	Octenol	15	8,460 ± 1,170 A
R-Octenol	20	8,340 ± 1,092 A	R-Octenol	15	8,062 ± 1,275 A
Red Blend	20	7,039 ± 1,606 A	Control	15	7,020 ± 1,179 A
Control	20	6,992 ± 1,156 A	Red Blend	15	6,824 ± 1,229 A

Within a column, means followed by the same capital letter designate no significant difference in collections among treatments (alpha=0.05).
"a" designates column for Study 1. "b" designates column for Study 2.
n = the number of trap replicates
degrees of freedom = 4, 99

was identified and counted. Samples were counted and identified to species using the keys of Blanton and Wirth 1979.

Data Analysis

Data were first normalized by conversion to square root then subjected to ANOVA (SAS 2003) using the following model statements: *C. furens* = Treatment trap night, where dependent variables represented numbers of *C. furens* captured; *C. mississippiensis* = Treatment trap night, where dependent variables represented numbers of *C. mississippiensis* captured. Treatment was one of the four lures (Red Blend, BG lure, octenol, R-octenol) or control, and trap night was one of the 100 trap nights of the study. Means were separated with the Ryan-Einot-Gabriel-Welsch Multiple Range Test (REGWQ), and unless otherwise stated, p-values were $P<0.05$ (SAS 2003). Although square root values were used for the analyses, actual values are reported in the text, figures and tables.

RESULTS

An estimated total of 1,426,830 *Culicoides* were collected during both study periods in Cedar Key, Florida and the LSNWR (Table 2). The vast majority of trap captures were due to two species, *C. furens* and *C. mississippiensis* (Table 3). *Culicoides melleus* (Coquillett) and *C. insignis* (Lutz) were also captured from Cedar Key and the LSNWR. However, these two species were quite rare and together only accounted for ~0.1% of the trap captures. Thus, we limited our analyses to the two most abundant species, *C. furens* and *C. mississippiensis.* Each of the two studies were conducted at different times of the year and at different locations, February to March 2009 on Cedar Key (Study 1) where *C. mississippiensis* was most prevalent and May-August 2009 at the Lower Suwannee National Wildlife Refuge (LSNWR) (Study 2) where *C. furens* was most prevalent. Given these substantial differences in species composition between the two sites and seasons of collection, we analyzed each study site separately.

Overall, we found no effect of lure on the number of *Culicoides* captured in either study but all lures increased trap capture above the control. In Study 1 on Cedar Key, there were no significant differences in the numbers of *C. mississippiensis* captured among lure treatments from February to March 2009. In Study 2 at the LSNWR, there were no significant differences in the number of *C. furens* captured among lure treatments from May to August 2009.

DISCUSSION

The trap sites chosen for this project produced two major *Culicoides* species (*C. furens and C. mississippiensis*) in the capture nets. In Study 2, the *Culicoides furens* population began to increase in April and was the dominant species for the LSNWR through the end of August. In Study 1, *Culicoides mississippiensis* was the dominant species from February to March in Cedar Key, FL but may be found in the capture nets throughout the entire study period (Lloyd 2006). *Culicoides melleus* and *C. insignis* were caught in lower numbers during the same trapping period; however, the lack of numbers in trap catch may be due to lack of attraction rather than lack of abundance. *Culicoides* seem to be selective and may have species-specific preferences when host seeking.

Furthermore, trap capture may not be an accurate depiction of *C. furens* or *C. mississippiensis* populations due to targeted host selection of *Culicoides* and lures selected for this study, which may only be a portion of the population.

Overall, there were no significance differences among treatments or locations when evaluated against number of host seeking *Culicoides* captured. The control treatment did not have a synthetic lure to mimic kairomones but the standard trap attractants (CO2, heat and moisture) seem to provide enough attraction to capture host seeking *Culicoides* in numbers that may be acceptable. However, octenol numerically outperformed other treatments when trapping *C. furens.* Similar selection preference was seen with *C. mississippiensis*, but this species preferred the BG lure over other treatments. The Red Blend treatment performed well in lab assays when evaluated against several mosquito species (Kline, personal communication); however, Red Blend was introduced in those studies by removing the lid from a container allowing it to evaporate freely, not released from a wick as in this study. Furthermore, Mann and Kaufman (2010) utilized aunique delivery method when targeting Phlebotomine sand flies in Florida. The authors placed a known amount of Red Blend on a cotton swab, placed the swab in a tube and suspended it from a trap at the CO2 outlet. To the author's knowledge, there is no standard delivery method for Red Blend and there is some concern with the delivery method used in this study and may be the cause of the poor attraction. The wick out method was consistent in the amount delivered per trapping repetition, but it may not be ideal for the intended target species; improper delivery of a lure may actually repel host seeking *Culicoides* rather than attract them to a trap (Afify and Potter 2020). Further analysis of delivery methods for the Red Blend lure should be investigated.

A higher number of *C. mississippiensis* were attracted to the BG lure rather than octenol. A review of the literature shows that two mosquitoes in the *Aedes* genera are attracted to BG lure (Kröckel et al. 2006, Obenauer et al. 2010) and some in the *Anopheles* genera prefer octenol over BG lure (Irish et al. 2008). Results in this study imply that there may be differences in attraction between *Culicoides* species as seen with mosquito species.

Although statistically insignificant, this study supports the findings of Kline and Wood (1988), Kline et al. (1994), and Kline and Lemire (1995) demonstrating *C. furens* attraction to octenol. In addition, *C. furens* expressed an elevated attraction to the other treatments (BG lure, R-octenol, and Red Blend) when compared to the control (no additional attractant) trap. Becker et al. (1995) reported that *Culex pipiens* did not display attraction to octenol and Burkett et al. (2001) reported that octenol actually decreased *Cx. pipiens* trap capture. Furthermore, Atwood and Meisch (1993) found that octenol alone had no effect on black fly trap capture. In this study, there may be a synergistic effectwith CO2 and BG lure or R-octenol as seen with CO2 and octenol synergism in previous studies (Atwood and Meisch 1993, Kline et al. 1994, Ritchie et al. 1994, Bishop et al.

2008). These additional lures may stimulate future research evaluating the effectiveness of multiple lures when combined with each other or in combination with additional cues, such as movement or heat.

Results from this study could not identify a lure with significant preference to *Culicoides* species captured in Cedar Key, Florida. There seems to be variation among species, even within the same genera, when utilizing cues to track hosts. Further studies evaluating species specific trap types as well as attractants are needed to develop an effective *Culicoides* adult trapping control system as part of an integrated biting insect control program.

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EFFECT OF NOZZLE ORIENTATION ON DISPERSION OF TRUCK MOUNTED ULTRA LOW VOLUME SPRAY AT DIFFERENT HEIGHTS AND DISTANCES[4]

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ABSTRACT

A field evaluation study was conducted to understand the dispersion of spray from a truck-mounted ultra-low-volume (ULV) sprayer when released with the nozzle oriented at the conventional angle of 45° in addition to 22° and 0° with respect to ground surface. The study was conducted in an open grassy field at the Anastasia Mosquito Control District by applying Aqualuer™ 20-20 at the highest label application rate. Mortality of caged female *Aedes aegypti* was tested at 1.5, 3.0, 6.0, and 8.5 m heights at 0, 30, 60, and 90 m from the spray line. Spray trials were conducted during early morning hours and all test groups were replicated three times over different days. During these evaluations higher mortality was recorded from the 22° and 0° nozzle orientations up to 30 m from the spray line and at heights up to 3 m. The conventional nozzle orientation of 45° provided the greatest mortality 30 m from application source at heights of 6.0 and 8.5 m.

Key Words: *Aedes aegypti*, nozzle angle, adulticide, droplet dynamics

INTRODUCTION

Ultra-low volume (ULV) space sprays of mosquito adulticides, in spite of their spatially short-lived duration of effectiveness, have no equal alternative when it is required to quickly and effectively suppress insect populations during public health emergencies. Perich et al. (2000) has described ULV as the best management practice against adult mosquito vectors during epidemics. The factors that cause environmental dilution (e.g., wind) of ULV application for area-wide control ultimately limit the temporal and spatial duration of toxicity against mosquito populations that result in not enough residual insecticide left behind. Consequently, inconsistent performance has been reported from a vast majority of studies evaluating ground ULV application of mosquito adulticides. These inconsistencies in control have been reported by some researchers as dismal performance (Reddy et al. 2006; Lesser and Latham, 2013; Xue et al. 2013), others showing excellent control (Mount et al. 1968; Stains et al. 1969; Taylor and Schoof 1971; Mount et al. 1978) while some others showing sporadic results (McNeill and Ludwig 1970; Mount et al. 1970; Rathburn and Boike 1975; Turner 1977; Bunner et al. 1987; Britch et al. 2011).

The effectiveness of mosquito adulticide applications in residential areas can be confounded by tree lines and other landscape features such as shrubbery, as well as physical structures that act as barriers to spray movement. Based on the density of these barriers, spray may pass through these barriers or may deflect over and around them. Realizing the complexities of the areas to be treated and inconsistencies in effectiveness of truck mounted ultra-low volume space sprays, a series of studies were conducted to investigate the causes of these inconsistencies. These studies were also aimed at optimization of spray applications and were conducted between 2014 and 2017. One study compared conventional 45° upward nozzle orientation with horizontal (0°) and 30° downward nozzle orientations in open field trials. Results revealed that horizontal nozzle orientation was most effective against caged female *Ae. aegypti* (L.) (Farooq et al. 2017). Another study (Farooq et al. 2018) assessed the effect of travel speed on spray effectiveness, also in open field settings and revealed that increased travel speed improved spray dispersion and effectiveness against caged female *Ae. aegypti*. Similar comparison in urban environments of Gainesville, FL indicated that horizontal nozzle orientation was a better alternative to the standard 45° upward nozzle orientation against caged female *Ae. aegypti* and *Ae. albopictus* (Skuse) (Jiang and Farooq 2016; Jiang et al. 2020). During these studies, effectiveness of spray was checked at 1.5 m above the ground. Effectiveness of sprays in all of the above-mentioned studies indicated that when spray nozzles were orientated horizontally, the spray plume remained centered around 1.5 m above ground surface. The pattern of spray movement during other orientations, e.g., 45° upward, was not evaluated. However, it remains unclear how spray plumes interact with residential vegetative barriers that can alter its effectiveness. To help resolve concerns about vertical drift of the spray plume, and along longitudinal distance, our objective was to evaluate spray dispersion at different heights and distances from the spray path when applied using three different nozzle orientations of a truck mounted ULV sprayer.

MATERIALS AND METHODS

Experiments were conducted at an adulticide evaluation field at the Anastasia Mosquito Control District (AMCD), St. Augustine, FL (N29.901119, W-81.413381). The application area measuring 122 m x 122 m is an open area grassy field bordered with slash pine trees (*Pinus elliotii*) and small shrubs along three sides and buildings on one side (Figure 1). During this study, Aqualuer 20-20 (AI: permethrin 20.6%, piperonyl butoxide 20.6%, Value Garden Supply, St. Joseph, MO) was applied using a truck mounted ULV sprayer (Guardian 190 ES, ADAPCO, Sanford, FL) at the maximum label rate. Nozzle orientations consisted of upward 45°, 22°, and horizontal (0°) positions in relation to ground surface. Each nozzle orientation was replicated three times.

Caged mosquitoes and droplet impinges (Leading Edge, Fletcher NC) were mounted at four different heights (1.5, 3.0, 6.0 and 8.5 m) on 8.5 m on telescopic poles at 0, 30, 61, and 91 m from the spray line (Figure 2A). At each height on the pole, cages and droplet impinges were on the opposite sides and 0.6 m away from the pole. Each cage held 25, 5-7 day old, laboratory reared insecticide susceptible (Orlando strain) females *Ae. aegypti*, that was attached to the rope at each location and height (Figure 2B). Similarly, spinning impinges were hung upside down

Figure 1: Experimental field shown on the map with trees visible on all three sides.

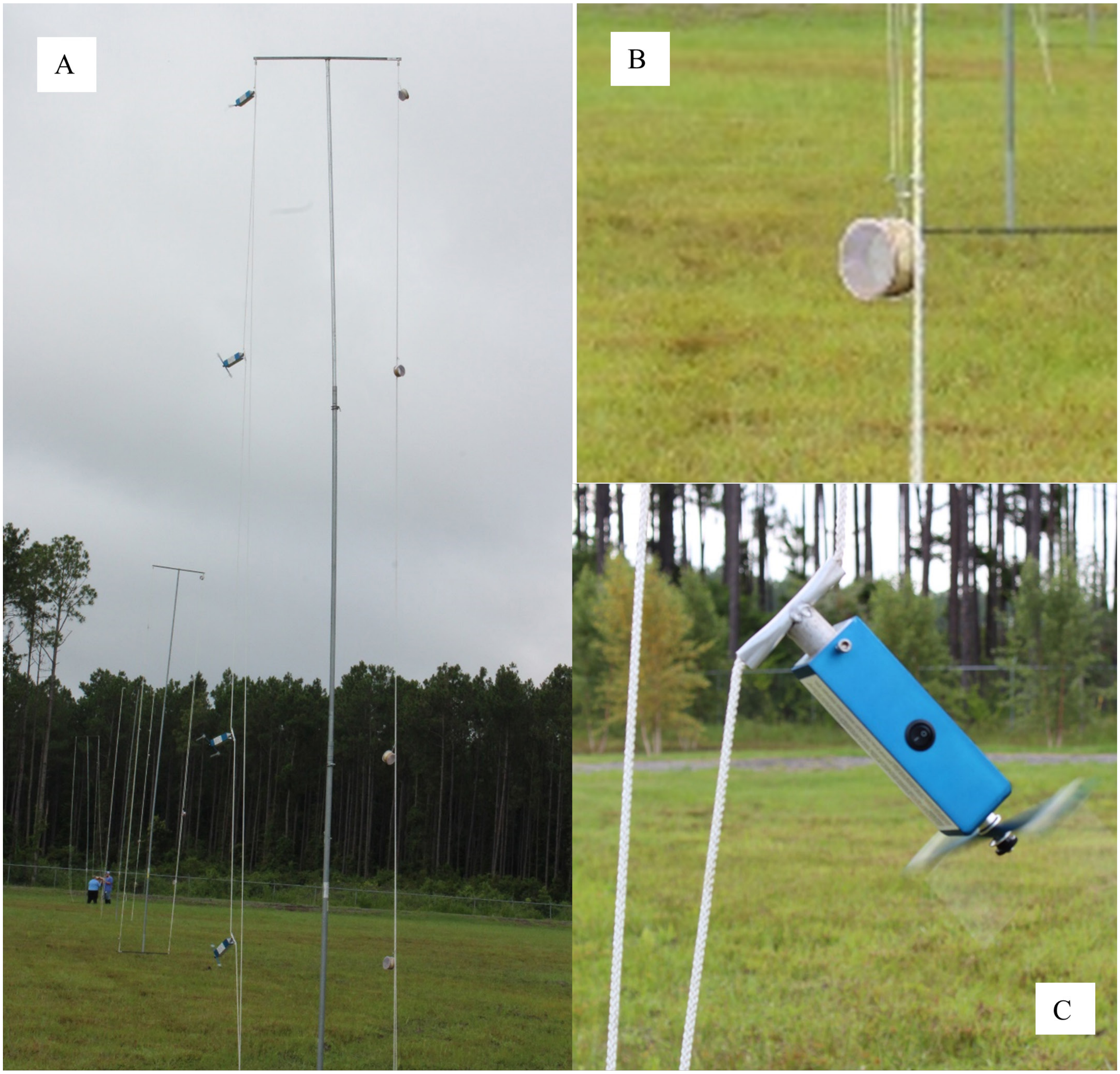

Figure 2: Field Layout. 2A) Overall view of the sampling locations showing relative position of cages and spinners; 2B) cages attached to the rope; 2C) spinner attached to the rope.

tied with a rope passing through a pulley at the end of the extension pipe (Figure 2C). Cage and impinger assemblies were raised before spray and lowered 15 min after the application. Each spinner had one Teflon® coated 3 mm rod for droplet size measurement (Leading Edge, Fletcher NC) and one plain rod to balance the spinner. After securing the slides and turning the spinner on, the cages and spinners were raised by pulling the rope. Fifteen minutes after the spray, the spinners and cages were lowered by releasing the rope and cages/slides were collected.

Three control cages were placed in the same outdoor environment for 15 minutes and removed before start of the spray. Applications were made with the drive path perpendicular to the sampling line when the wind direction would carry drift towards the cages. For each treatment pass, spray was initiated 30 m before reaching the sampling line and continued about 30 m past the line. One replication of each nozzle orientation was completed in one day and the experiment spanned over three weeks. Applications were made between 0800 and 1100 under slightly convective and neutral conditions. It has been

Table 1: Ranges of weather conditions during all the spray applications

Treatment	Wind Speed km/h	Wind Direction, Angle (°) w. r. t. Sampling Line	Temperature °C	Relative Humidity %
Horizontal Nozzle	4.8 – 9.7	30.0	25.6 – 32.2	59.5 – 69.0
Nozzle 22° Upward	6.4 – 8.0	30.0 – 60.0	28.9 – 33.4	50.7 – 67.7
Nozzle 45° Upward	1.6 – 9.7	0.0 – 60.0	30.6 – 31.9	61.7 – 67.4
Control	1.6 – 9.7	30.0 – 60.0	25.2 – 28.8	60.0 – 73.7

demonstrated in a previous study that when wind speed is > 5 mph, inversion is not required for a ULV application (Miller et al. 2012). Wind speed, wind direction, temperature and relative humidity were recorded at 1.5 m above ground with WatchDog 2550 weather station (Spectrum Technologies Inc., Plainfield, IL). The weather data is summarized in Table 1.

Fifteen minutes after application, cages were removed and taken to the laboratory, provided with 10% sugar solution, and stored under ambient laboratory conditions. Mortality was observed at 1 and 24 h after treatment. Rods from spinners were removed at the same time. Teflon® coated rods were stored in pre-labeled boxes for droplet measurements which were completed within 24 h after spray application.

The control mortality in all cages was from 0-4 % with an average of 1.5% and the mortality was not adjusted for control mortality. An analysis of variance was performed on 1 and 24 h mortality using JMP Version 14 (SAS Institute Inc, Cary, NC). Mean mortality between heights and distance separately were compared with Tukey's multiple comparison test and were considered significant at $p<0.05$. As there were considerable number of rods which did not have enough droplets to measure, the available data for droplets was not meaningful and is not reported.

RESULTS

Ae. aegypti mortality was recorded at both 1-h and 24-h post-treatment. However, because mortality at 1-h and 24-h post-treatment was consistent, only 24-h post-treatment data is presented. Analysis of variance indicated that *Ae. aegypti* mortality at 24-h post-treatment was not significantly affected by nozzle angle ($F = 0.11$, $df = 2$, $p = 0.89$) and height ($F = 0.79$, $df = 3$, $p = 0.5$). However, the mortality was significantly affected by distance from the spray line ($F = 5.47$, $df = 3$, $p = 0.0013$). The interaction of spray distance, height, and nozzle orientation did not have a significant effect on 24 h mortality. The 24 h mortality, was greater at distance 0, at height of 1.5 and 3.0 m above ground, from 0° and 22° nozzle orientations compared with 45° orientation (Fig 3). At 6.0 and 8.5 m heights, 45° nozzle orientation resulted in greater mortality than the other two orientations. These trends indicated that spray using nozzles at 0° and 22° orientations remained close to the ground while spray using nozzle at 45° orientation moved to the upper heights. At 30 m distance from application path, the 0° and 22° orientations had high mortality compared to 45° orientation. At other distances, the 0° resulted in lower mortality compared with 22° and 45° orientations.

DISCUSSION

In this study, we found that spray from the horizontal (0°) nozzle orientation showed greater mortality near the spray line, but resulted in less mortality at farther distances when compared to the 22° and 45° orientations. This result generally contradicts to what has been observed in prior studies (Farooq et al. 2017 and Jiang et al 2020) which showed higher mortality from horizontal nozzle at all distances. A key difference in our study was that the application site (while in an open field) was surrounded by forest on three sides (Figure 1). When the wind crosses over the tree line, it creates a downdraft with eddies near the trees as shown in Figure 4 (Iowa State University). This downward movement of wind pushes the spray plume towards the ground. This movement could be substantive even in low wind speeds as the ULV droplets are too small to resist air movement. The results of our study show that this downdraft phenomenon was a factor to depress the expected upward range of ULV drift. We believe that the spray was pushed down by the air current coming from top of the trees that negatively affected the 0° spray application where droplets were pushed below the level of mosquito cages in the field showing lower mortality. The downdraft phenomena also corrected the trajectory of the 45° sprays, resulting in greater-than-expected mosquito mortality. This phenomenon may affect the spray more in the morning (when this study was conducted) when strong inversion conditions developed overnight still existed. In the evening, the inversion is just in its inception and its effect is reduced. Therefore, it is important that the time in which an application is performed can considerably affect the effectiveness of a treatment. In an experiment to study the potential of ULV spray to penetrate dwellings to control dengue vectors harboring indoors, it was found that horizontal nozzle orientation resulted in considerable concurrent mortality of *Ae. aegypti* inside and outside of the dwelling (Farooq et. al 2020). The horizontal nozzle orientation by keeping spray close to the ground also provides an opportunity

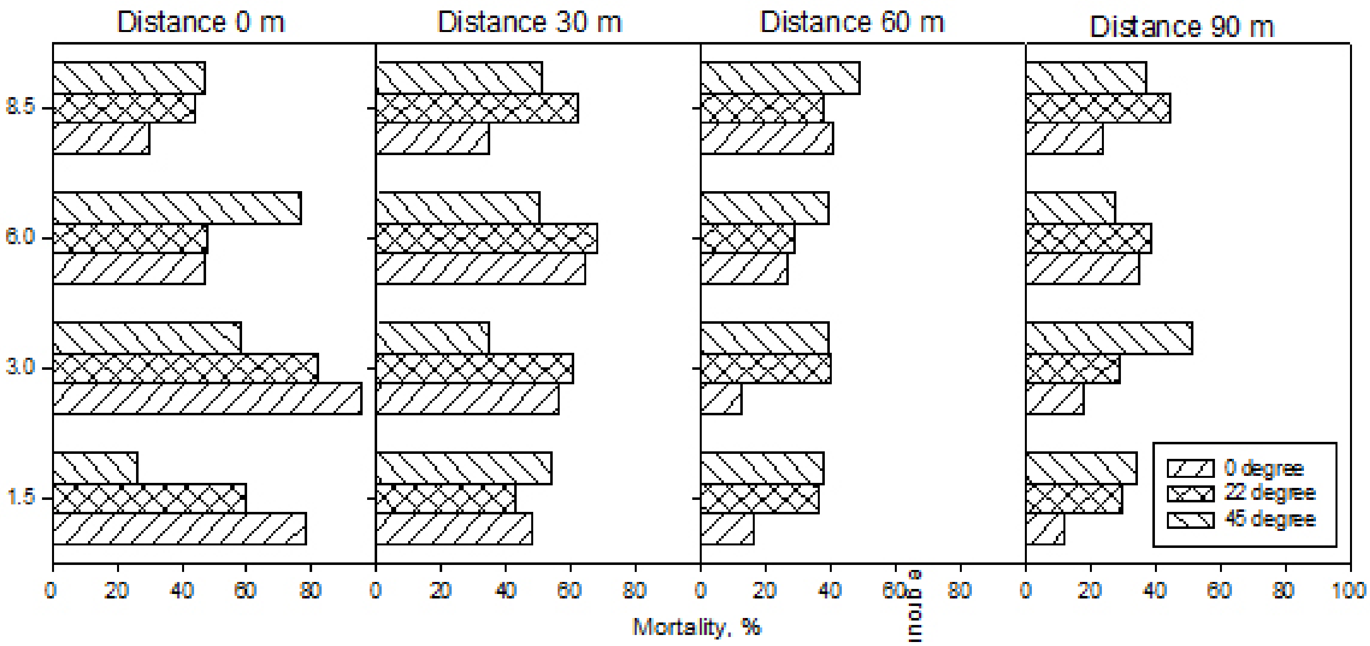

Figure 3: Mosquito mortality from spray delivered with nozzle at different orientations to different distances and heights

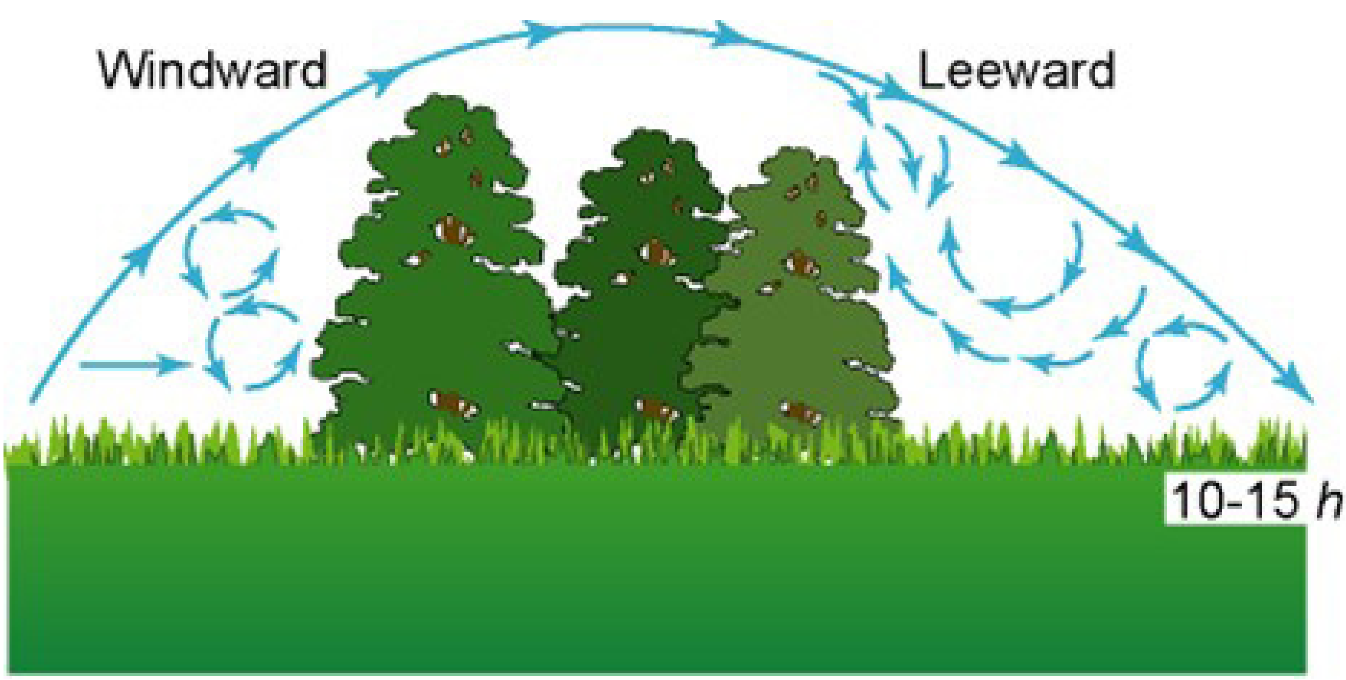

Figure 4: Wind Profile over trees (Courtesy: Iowa State University)

to target mosquitoes hiding under vegetation and raised buildings but it needs to be further investigated. Results of this and other studies on this topic indicate the need to adjust the nozzle orientation to suite the habitation being treated.

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LABORATORY EVALUATION OF BIGSHOT MAXIM, REPELCARE, AND CLOVE ESSENTIAL OIL, *SYZYGIUM AROMATICUM* AGAINST THE LONE STAR TICK, *AMBLYOMMA AMERICANUM*

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ABSTRACT

Synthetic acaricides have been the most used method for controlling tick populations. However, their frequent application has had negative impacts on treated animals and the environment and led to the development of resistance in tick populations. These factors generated interest and the need to find more environmentally friendly alternatives. In this study, the repellency and acaricidal effects of BigShot Maxim (AIs: cedarwood oil, cinnamon oil, thyme oil), RepelCare (AIs: turmeric oil and eucalyptus oil), and clove oil, *Syzygium aromaticum* L., were tested against the lone star tick, *Amblyomma Americanum* (Linnaeus). Despite no evidence of repellency for BigShot Maxim, RepelCare and clove oil, its disruption of the host seeking behavior after contact to the products was observed and further investigated. BigShot Maxim and clove oil were selected for mortality testing and resulted in complete mortality of male, female ticks, and nymphs with some application rates. The results from this study provide a better understanding of repellency and acaricidal effects of botanical products against the lone star tick that can be used to further improve the development of green chemistry. However, further studies are warranted before these botanical products can be implemented as effective alternatives to chemical acaricides to use in integrated vector control.

Key Words: essential oils, tick, repellent, mortality

INTRODUCTION

Ticks transmit more pathogen species than any other blood-feeding arthropods in the United States (US) (Nicholson et al. 2019) and spread diseases such as Lyme disease, ehrlichiosis, Rocky Mountain Spotted Fever, Southern Tick-Associated Rash Illness (STARI), babesiosis, etc. During 2004 - 2016, tick-borne diseases had more than doubled and accounted for 77% of all vector-borne diseases reported (Rosenberg et al. 2018). The lone star tick, *Amblyomma americanum* (Linnaeus), are the most reported ticks to bite humans in the southeastern and south-central US (Masters et al. 2008). Until the 1990s, the lone star tick was thought of as a nuisance species and poor vector of diseases affecting humans. However, the lone star tick has been identified to transmit several pathogens and, under suitable environment, can transmit diseases such as ehrlichiosis, rickettsiosis, tularemia, and protozoan infections (Childs et al. 2003; Goddard and Varela-Stokes 2009; Eisen et al. 2017b). With the lone star tick being an aggressive feeder, having a wide geographic range, and high population densities, it is a public health concern and has been directly linked to the increase in incidence of tick-borne diseases.

Tick control and bite prevention are crucial parts of strategies to prevent the spread of tick-borne diseases. Chemical acaricides and repellents have had a vital role in these efforts as being the most effective tools against ticks (Scott et al. 2016). The types of chemicals available on the market for protection against ticks are very limited and the primary chemicals used are *N*, *N*-diethyl-3-methylbenzamide (DEET) and permethrin (Meng et al. 2016). Unfortunately, the intensive use of chemicals has resulted in populations of ticks being resistant to these chemicals (Rodriguez-Vivas et al. 2017; Selles et al. 2021). Not only was there concern about insecticide resistance, but there was also a growing fear for environmental impact and public apprehension to use these chemicals due to the perception that synthetics are harmful. This heightened the interest in sustainable chemistry to find safe and effective alternatives to these synthetic insecticides for tick control and bite prevention.

Fortunately, the United States Environmental Protection Agency (EPA) allows for minimum risk pesticides to be exempt from having to be registered with the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). To be considered a minimum risk pesticide, the product must only contain active and inert ingredients that fall under 40 CFR 152.25(f)(1). This exemption encouraged the research and development of sustainable products, such as pesticides containing essential oils.

Many essential oils have been tested against ticks and have shown potential to be alternatives to synthetic acaricides (Bissinger and Poe 2010; Carroll et al. 2010; Jordan et al. 2012; Bissinger et al. 2014). The goal of this study was to evaluate two formulated products containing essential oils as active ingredients and clove essential oil, *Syzygium aromaticum* L., as an alternative to synthetic repellents and acaricides against *A. americanum.*

METHODS AND MATERIALS

Ticks. Male, female and nymphs of *A. americanum* ticks were obtained from Oklahoma State University Department of Entomology, Stillwater, OK, U.S.A. and were kept in Anastasia Mosquito Control District (AMCD) insectaries maintained at 26.6° ±1° C and 70-80% relative humidity. The study tested three repellents: (1) BigShot Maxim (PreVasive USA Oakwood, GA) which contains cedarwood oil (14%), thyme oil (0.53%), and cinnamon oil (0.23%), (2) RepelCare (PerdYaThai Industry, Pharnomsarakam, Thailand) containing turmeric and eucalyptus oil, and (3) pure clove essential oil.

Repellency Test. For repellency evaluation, procedure outlined by Farooq et al. (2022) was adapted. For these tests, a 7.5 cm outer band of a 30.0 cm diameter plastic plate was sprayed, while a 15.0 cm diameter inner circle was left unsprayed. To do this, a 15.0 cm diameter plate was placed in the middle of the 30.0 cm diameter plate and the product was sprayed using a Master Airbrush model G22 (Master Airbrush, G22, China) with a portable mini air compressor model C16-B (Master Airbrush, C16-B, China). The applications were made at specified rates of 8.3, 16.7 or 25 mL/m^2 of the surface area with the spray brush, referred to as low, medium, and high rates for all repellents. The surface area of the plates used was 720 cm^2, so 0.6-, 1.2-, and 1.8-mL product was sprayed per plate with three plates per application rate to form three replicates. Three plates were also treated with water at 16.7 mL/m^2 as the control. One repellent was evaluated on one day and separate controls were used for each repellent. The BG Lure (Biogents, Regensberg, Germany) has been shown to be an attractant for the lone star tick (Farooq et al. 2021) and its grains were placed around the large plate in groups of 10 at 8 spots in a circle to create an attraction for the ticks as shown in Figure 1.

A group of five male or female ticks, selected randomly for each replicate plate, were released in the middle of the plate on the untreated surface, and their movement was observed for 15 minutes. Any ticks that crossed the treated surface and went out of the plate were collected and stored for mortality observation assuming that the ticks picked up some of the product while walking over the treated surface. After 15 minutes, the number of ticks that crossed the treated surface, remained on the treated surface, or remained in the untreated circle were recorded separately. Behavior of the ticks in the form of (a) movement out of the plate, (b) remaining on treated surface and (c) remaining on the untreated surface, collectively called dispersion of ticks to these locations. The percent of ticks that remained in the inner untreated circle was considered as repelled. After the first run, another group of five ticks of the opposite sex from the ones used in the prior run, were released on the same plate respectively. All ticks after each run were collected and stored in vials for 24 hrs after exposure to the insecticide and then mortality was recorded. For mortality determination, any tick that was immobilized was poked with forceps multiple times to confirm its immobility and only ones that failed to move were considered as dead and included in the mortality.

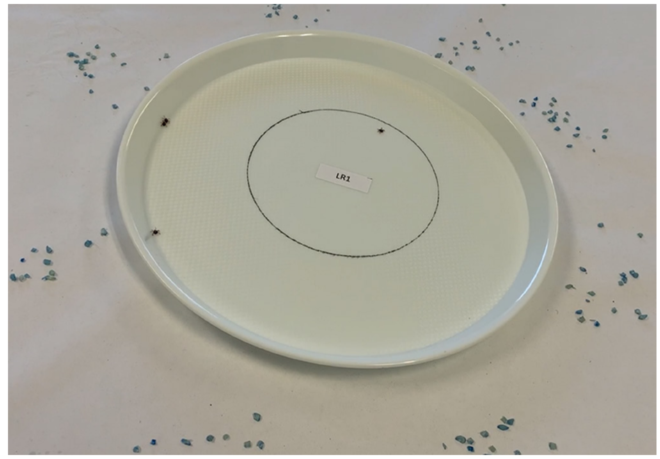

Figure 1. Setup for tick repellency test.

Toxicity Test. To evaluate as an acaricide, *A. americanum* were exposed to three application rates of either Bigshot Maxim or clove oil. RepelCare was not evaluated since there was no mortality recorded from exposure to this product during the repellency test while other two repellents resulted in some mortality of ticks (Table 1). For this test, 14-cm diameter petri dishes were sprayed using the rates of 8.3, 16.7 or 25 mL/m^2 via Master Airbrush model G22 with a portable mini air compressor model C16-B and covered with a petri lid. For this dish size, 0.25 mL of product was sprayed per mL of application rate through the airbrush. The spray was allowed to dry for 10-15 minutes or until no visible droplets on the petri surface. Control dishes were sprayed with only water. One product was evaluated in one day and separate controls were used for each product. For each dish, five ticks were released into the dish and covered with the respective petri lid. After 30 minutes, the ticks were removed from the dishes and placed into clean vials. The vials were maintained under room temperature and mortality was recorded at 72 hrs after exposure. After removing all the ticks, dishes were stored at room temperature for retesting after one day and two days after treatment, and then every week after treatment using a new batch of each tick sex and stage until mortality declined to below 50%.

In the first test, plastic dishes were used and only the bottom dish was sprayed. It was noted that after releasing ticks and covering the dish, the ticks spent some time crawling on the underside of the cover. In addition, BigShot Maxim reacted with the plastic and caused the dish to change from transparent to opaque (Figure 2). To overcome this, glass petri dishes were obtained and used instead of plastic petri dishes to prevent any influence that may occur from the reaction. Also, both the tops and bottoms of the petri dishes were sprayed at the same rate by applying double the amount of the spray. The first replication was repeated with the glass petri dishes.

Data Analysis. The normality test of the repellency and mortality data found it to have non-normal distribution and Wilcoxon test of nonparametric analysis was performed to assess the significance of difference between different repellents and control and between different rates of each repellent and control using JMP edition 15. A P-value of less than 0.05 indicated statistical significance. The pairwise means were compared using Wilcoxon Each Pair comparison using nonparametric multiple comparison function at 95% level of significance using JPM 15. The mortality data for nymphs indicated higher than acceptable control mortality. Thus, the mortality of nymphs was corrected using Abbotts formula (Abbott 1987).

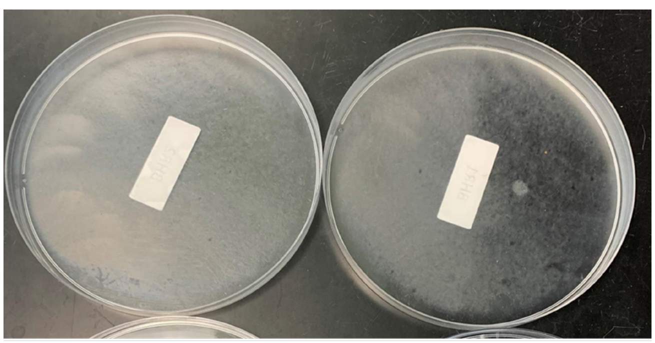

Figure 2. Reaction on plastic petri dishes caused by spraying BigShot Maxim.

RESULTS

Repellency Test. The overall effect of repellents on dispersion of ticks between different test areas and their mortality is shown in Table 1. There was no effect of sex on percent of ticks in any section (χ^2<0.6, df=1, p>0.4) and the data for both sexes was combined. Overall, the percentage of ticks that remained inside the circle was not affected by the repellents being tested or control ((χ^2=5.6, df=3, p=013.4) and there also was no effect of repellents on percent of ticks that crossed the surface treated with repellents ((χ^2=4.2, df=3, p=0.237). On the other hand, the percent of ticks that remained on treated surface were significantly affected by the repellent ((χ^2=17.0, df=3, p=0.0007). Also the tick mortality was significantly affected by repellent (χ^2=26.6, df=3, p<0.0001). As shown in table 1, more ticks stayed on treated surface with BigShot Maxim and clove oil compared to surfaces treated with RepelCare and water. The data also showed that more ticks died due to interaction with these two repellents than RepelCare and water.

The effects of three rates of each repellent in comparison with each other and with control are given in table 2. In BigShot Maxim tests, the percentage of ticks in the untreated circle was not significantly affected by rate (χ^2=2.5, df=3, p=0.475) and was not different than water, which indicates that the ticks did not show spatial repellency. The rates of BigShot Maxim significantly affected percent of ticks on treated surface (χ^2=14.8, df=3, p=0.002), the percent of ticks that crossed the treated surface (χ^2=13.0, df=3, p=0.004), and tick mortality (χ^2=14.7, df=3, p=0.002). With the lowest rate of BigShot Maxim, ticks did not stay on the treated surface and almost all crossed the surface, whereas for medium and high rate, significantly higher percentage stayed on treated surface. That behavior indicated that ticks were physically affected by these rates of repellent which blocked their attraction to the attractant outside of the treated surface and their staying longer on treated surface resulted in higher mortality with these two rates. In clove oil tests, the percentage of ticks in the untreated circle was not significantly affected by rate (χ^2=2.9, df=3, p=0.398) and was not different than water, which indicates that the ticks did not show spatial repellency. The rates of clove oil significantly affected percent of ticks on treated surface (χ^2=7.5, df=3, p=0.057), the percent of ticks that crossed the treated surface (χ^2=7.6, df=3, p=0.055), and tick mortality (χ^2=13.7, df=3, p=0.003). With all rates of clove oil except medium, most of the ticks crossed the surface whereas with medium rate significantly higher percentage stayed on treated surface. That behavior indicated that ticks were physically affected by this rate of repellent

Table 1. Mean percent dispersion of adult ticks for different products.

Product	Mean % Ticks (± SE) at different sections of the plate			
	Inside circle	On insecticide	Crossed insecticide	Mortality
Water	12.2 ± 4.9 AB	24.4 ± 3.8 AB	63.3 ± 5.9 A	1.1 ± 1.1 B
BigShot Maxim	4.4 ± 3.5 B	37.8 ± 7.2 A	57.8 ± 7.6 A	41.1 ± 10.2 A
Clove Oil	12.2 ± 3.7 A	30.0 ± 4.9 A	57.8 ± 5.1 A	41.1 ± 10.9 A
RepelCare	18.9 ± 5.9 A	7.5 ± 3.6 B	73.7 ± 6.7 A	0.0 ± 0.0 B

Table 2: Mean percent dispersion adult ticks at different rates of BigShot Maxim and clove oil.

Product	Rate	Mean ticks (% ± SE)			Percent Mortality
		In Untreated Circle	On Treated Surface	Crossed Treated Surface	
BigShot Maxim	Low	0.0 ± 0.0 A	3.3 ± 3.3 B	96.7 ± 3.3 A	6.7 ± 4.2 B
	Medium	10.0 ± 10.0 A	56.7 ± 10.9 A	33.3 ± 8.4 B	30.0 ± 13.4 B
	High	3.3 ± 3.3 A	53.3 ± 6.7 A	43.3 ± 6.1 B	86.7 ± 13.3 A
	Control	20.0 ± 12.7 A	26.7 ± 8.4 AB	53.3 ± 13.3 B	3.3 ± 3.3 B
Clove Oil	Low	16.7 ± 6.1 A	16.7 ± 9.9 B	66.7 ± 9.9 AB	0.0 ± 0.0 B
	Medium	10.0 ± 6.8 A	46.7 ± 6.7 A	43.3± 3.3 B	76.7 ± 15.8 A
	High	10.0 ± 6.8 A	26.7 ± 8.4 AB	63.3 ± 9.5 AB	46.7 ± 19.1 AB
	Control	3.3 ± 3.3 A	23.3 ± 6.1 B	73.3 ± 6.7 A	0.0 ± 0.0 B
RepelCare	Low	20.0 ± 13.7 A	5.7 ± 3.7 B	74.3 ± 12.4 A	0.0 ± 0.0
	Medium	13.3 ± 8.4 A	0.0 ± 0.0 B	86.7 ± 8.4 A	0.0 ± 0.0
	High	23.3 ± 9.5 A	16.7 ± 9.5 AB	60.0 ± 12.6 A	0.0 ± 0.0
	Control	13.3 ± 6.7 A	23.3 ± 6.1 A	63.3 ± 9.5 A	0.0 ± 0.0

which blocked their attraction to the attractant outside of the treated surface and their staying longer on treated surface resulted in higher mortality with this rate. With the high rate, despite less ticks staying on treated surface, the mortality was considerable. In RepelCare tests, the percentage of ticks in the untreated circle and percentage of ticks that crossed treated surface were not significantly affected by rate (χ^2=0.9, df=3, p=0.814 and χ^2=4.0, df=3, p=0.257, respectively). The rates of RepelCare significantly affected percent of ticks on treated surface (χ^2=9.3, df=3, p=0.026) but all the numbers were low. In RepelCare tests, tick mortality was none. With all rates of RepelCare, most of the ticks crossed the surface indicating that their response to attractant outside of treated surface was not affected by the presence of repellent indicating lack of repellency to these ticks.

Toxicity Test. Mortality of ticks was an indicator of toxicity and the data indicated that the repellents had a significant effect on growth stages of ticks by BigShot Maxim (χ^2=9.0, df=2, p=0.02) and clove oil (χ^2=10.7, df=2, p=0.005). Up to day 2, from both BigShot Maxim and clove oil, the nymphs had overall higher mortality (76.5 ± 6.1 and 68.6 ± 7.1 %) compared to male (54.0 ± 7.4 and 47.1 ± 7.8 %) and female (48.6 ± 7.7 and 37.7 ± 7.3 %) ticks. Thus, further analysis was split by male, female, and nymphs. For males, females, and nymphs, the difference in overall mean mortality from BigShot Maxim and clove oil was not significant ((χ^2=0.5, df=1, p=0.48; χ^2=1.0, df=1, p=0.33; χ^2=2.6, df=1, p=0.11, respectively).

Table 3 shows the mean percent mortality of male ticks for each product at different rates and residual control in days after treatment. Medium and high rates of BigShot Maxim resulted in significantly higher mortality of male ticks than control and low rate. The low rate never had significantly higher mortality than control and can be ruled out. Middle rate of BigShot Maxim was effective up to one day after treatment, while high rate was effective up to two days after treatment. As indicated by data in Table 3, low rate of clove oil had some male tick mortality on day 0 but not on other days. The effectiveness of medium and high rates of clove oil lasted up to one day and both were not effective on second day. Table 4 shows mean percent

Table 3: Mortality of male ticks at different rates of BigShot Maxim and clove oil on different days after application.

Product	Rate	Mean mortality (% ± SE) of male ticks days after application		
		Day 0	Day 1	Day 2
BigShot Maxim	Low	66.7 ± 17.6 AB*	33.3 ± 13.3 AB	0.0 ± 0.0 C
	Medium	94.4 ± 5.6 AB	100.0 ± 0.0 A	60.0 ± 0.0 B
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	93.3 ± 6.7 A
	Control	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 C
Clove Oil	Low	78.3 ± 11.7 AB	33.3 ± 17.6 AB	0.0 ± 0.0 A
	Medium	100.0 ± 0.0 A	93.3 ± 6.7 A	0.0 ± 0.0 A
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	60.0 ± 30.6 A
	Control	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 A
*Means in a column for each product with same letter are not significantly different (α = 0.05)				

Table 4: Mortality of female ticks at different rates of BigShot Maxim and clove oil on different days after application.

Product	Rate	Mean mortality (% ± SE) of female ticks days after application		
		Day 0	Day 1	Day 2
BigShot Maxim	Low	93.3 ± 6.7 AB*	46.7 ± 17.6 AB	0.0 ± 0.0 A
	Medium	100.0 ± 0.0 A	93.3 ± 6.7 AB	33.3 ± 17.6 A
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	16.7 ± 16.7 A
	Control	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 A
Clove Oil	Low	33.3 ± 17.6 AB	5.6 ± 5.6 B	0.0 ± 0.0 A
	Medium	86.7 ± 13.3 AB	86.7 ± 6.7 A	0.0 ± 0.0 A
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	40.0 ± 11.5 A
	Control	0.0 ± 0.0 B	0.0 ± 0.0 B	0.0 ± 0.0 A
*Means in a column for each product with same letter are not significantly different (α = 0.05)				

Table 5: Abbott corrected mortality of nymphs at different rates of BigShot Maxim and clove oil on different days after application.

Product	Rate	Mean mortality (% ± SE) of nymphs days after application				
		Day 0	Day 1	Day 2	Day 7	Day 14
BigShot Maxim	Low	100.0 ± 0.0 A*	84.6 ± 15.4 A	72.9 ± 14.6A	86.7 ± 6.7 A	30.8 ± 23.1 A
	Medium	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A	93.3 ± 6.7 A	12.8 ± 9.2 A
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A	86.7 ± 6.7 A	69.2 ± 7.7 A
	Control	26.7 ± 13.3 B	13.3 ± 13.3 B	20.0 ± 11.5 B	0.0 ± 0.0 B	13.3 ± 6.7 A
Clove Oil	Low	100.0 ± 0.0 A	11.3 ± 5.9 B	76.7 ± 14.5AB	64.7 ± 7.1 A	36.3 ± 21.1 A
	Medium	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A	50.6 ± 18.7 A	19.0 ± 19.0 A
	High	100.0 ± 0.0 A	100.0 ± 0.0 A	100.0 ± 0.0 A	24.3 ± 17.2 A	23.8 ± 11.9 A
	Control	28.3 ± 6.0 B	6.7 ± 6.7 B	0.0 ± 0.0 B	5.6 ± 5.6 A	6.7 ± 6.7 A
*Means in a column for each product with same letter are not significantly different (α = 0.05)						

mortality of female adult ticks for different rates of each product and residual control in days after treatment. The low rate of BigShot Maxim had good female mortality on day 0 but it declined quickly after that. The medium and high rates were effective up to day one and then reduced to control level. The low rate of clove oil did not have enough mortality at any day (Table 4). However, medium, and high rates had considerable mortality up to day one.

The toxicity results for nymphs showed some mortality from control which might have been the result of handling nymphs during this study due to their size and soft bodies. Thus, nymphal mortality was adjusted for control mortality. Nymphal mortality from all rates of BigShot Maxim, as shown in Table 5 was significantly higher than control up to day 7 after treatment but was not significantly different from each other. On day 14, none of the rates produced nymphal mortality significantly higher than control. Low rate of clove oil resulted in significantly higher nymphal mortality than control on day 0 only (Table 5). The medium and high rates produced significantly higher nymphal mortality than control up to day 2 after treatment which indicates that it is less stable than BigShot Maxim. On day 7 and 14 after treatment, all rates of clove oil produced similar mortality as control (Table 5).

DISCUSSION

In this study, botanical products were evaluated for repellency against *A. americanum*. The results from the repellency test indicate that none of the products had repellency effects on adult ticks. However, it is argued that the characterization of repellents should differ between flying arthropods and crawling arthropods. Ticks have shown to display various reactions against chemical products, such as the 'hot foot' effect, anti-attachment, or disruption of attachment (Halos et al. 2012; Eisen et al. 2017a). Examples of these include ticks falling off soon after coming in contact with the product, lack of movement, and even the detachment of already attached ticks (Schreck et al. 1978; Ian and Bryan 1981; Lane 1989; Dryden et al. 2006).

During this study, it was observed that the ticks that moved over the treated surface at the beginning had mobility reduced with time. It could be assumed that there was some sort of effect after contacting the product based on higher percentage of ticks on treated surface at medium and higher rates of BigShot Maxim and clove oil than control, and their respective low rates (Table 2). This understanding is also supported by considerable mortality of ticks exposed to these two products during repellency testing (Table 2). The data does not show that trend for RepelCare as most of the ticks exposed to this product crossed the treated surface, left the test area, and survived. Since host-seeking ticks respond to certain host odors, it is possible that these products after having contacted ticks during crawling over treated surface may have masked the attractant odor. Thus, although there was no true repellency shown, it may have been possible there was a disruption in the host-seeking behavior or a disorientation in the tick's behaviors such that it still provides protection through the prevention of attachment (Jaenson et al. 2005; Pålsson et al. 2008). Also, the ticks exposed to Bigshot Maxim and clove oil during repellency testing indicated considerable mortality. As a result, it is expected that the ticks, even if they encounter human skin treated with these products, their presence on the skin will be limited due to decreased mobility and ultimate mortality, which will prevent them from attaching to the body. This is an indirect way of protection against the ticks.

Since the duration of product exposure for each tick varied in the repellency study, the mortality taken was used as a preliminary indicator for the products used in the toxicity test. Thus, only BigShot Maxim and clove oil were selected to do further toxicity testing as RepelCare did not result in any mortality during repellency testing.

The mortality study analysis indicated varied effects of repellents on male and female adult and nymphal life stages. Against adult male ticks, the results indicated that the high rate of BigShot Maxim was most effective and showed highest mortality than any rate of BigShot Maxim or clove oil with >90% mortality up to day two after treatment (Table 3). Meanwhile, the medium rate of both products was effective with >90% mortality up to day one against male adult ticks. For female ticks, the results indicated that the medium and high rate of BigShot Maxim were effective with >90% mortality up to day one after treatment (Table 3). Whereas only the high rate of clove oil was effective with >90% mortality up to day one after treatment. Lastly, against the nymphal stage, the medium rate of BigShot Maxim was most effective than clove oil with >90% mortality up to seven days after treatment. The differences in efficacy may be due to the susceptibility of the ticks at different life stages. In a systematic review by Nwanade et al. (2020), they focused on articles published between January 2017 and November 2019 using botanical acaricides and repellents against ticks. They observed that the nymphal stage of ticks was more vulnerable to products than adult ticks in many of the studies (Nwanade et al. 2020). This is due to the susceptibility of ticks being related to the thickness of the cuticle which only occurs after nymph ecdysis and becomes more pronounced in adult ticks (Adenubi et al. 2018).

Overall, BigShot Maxim, clove oil, and RepelCare did not have any repellency effect against *A. americanum*. On the other hand, both BigShot Maxim and clove oil did prove to have an acaricidal effect resulting in up to 100% mortality with some rates. The results indicated that the medium rate of BigShot Maxim was the optimal rate for mortality against these ticks. It showed to be >90% effective against female and male adult ticks up to one day after application and >90% effective against nymphal ticks up to seven days after application. The results also indicated medium rate of clove oil as the optimal for mortality against ticks tested. It showed to be >90% effective against female and male adult ticks up to one day after application and >90% effective against nymphal ticks up to two days after application. The limited residual effects were a result of the product containing essential oils which are highly volatile (Koul et al. 2008; Salman et al. 2020; Selles et al. 2021).

Insecticide resistance continues to be a growing problem with the chemical-based anti-tick measures (Adenubi et al. 2016). Fortunately, botanical products provide an alternative to combat insecticide resistance and control for resistant species while allowing for safer reapplications with minimal impact to the environment because of the volatility of essential oils. With the progress made in green chemistry, there is incentive to incorporate botanicals into integrated vector management. This study provided a better understanding of the acaricidal effects and repellency of these products against *A. americanum*. Further research efforts should be geared towards product contact assay, toxicity side effects on humans, animals and/or non-target organisms, and field control studies before being implemented into integrated vector management.

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EFFICACY TRIALS AND PERFORMANCE EVALUATION OF FIVE FLEA TRAPS IN BAMAKO, MALI

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ABSTRACT

Five off the shelve commercially available flea traps were tested for their efficacy against the cat/dog flea *Ctenocephalides felis*. Two traps were significantly better at catching fleas than the remaining three which performed very poorly. It is presumed that the unique combination of attractive features of the best performing Flea Catcher trap are what makes it so effective, including three large, intermittently illuminated light panels simulating host movement and additional synchronized lights in green wavelength pointed downwards to the flea catching base. Additionally, the Flea catcher has the largest surface area. Only two traps, the Flea Catcher and myFleaTrap were non-attractive to non-target insects, while the other traps caught large amounts of other insects quickly blocking the glue panels.

Key Words: *Ctenocephalides felis*, attractive features, flea traps, intermittent light, 510-550 nm light, non-target insects.

INTRODUCTION

The biting flea *Ctenocephalides felis*, also known as the "cat flea", is a major nuisance to dogs and cats as well as their owners by causing flea-related dermatitis (Rust and Dryden, 1997) as well as several diseases of medical and veterinary importance. *C. felis* transmits *Dirofiliria immitis* (dog tapeworm; Rust 2017) and the rickettsial diseases *Rickettsia felis* (Nelson et al. 2018; flea-borne spotted fever) and *Rickettsia typhi* (Blanton et al. 2016; murine typhus) (Bitam et al. 2010; Rust 2017).

Cat flea infestations on dogs and cats can be treated using oral or topical chemicals such as methoprene and pyriproxyfen, fipronil and imidacloprid respectively (Rust 2010). Topical products do not always stop fleas from engorging and causing flea allergy dermatitis and this was recognized as a reason for trapping before fleas get on the host (Dryden 2009). There are, however, problems with using chemicals to control fleas. For example, even though fipronil and imidacloprid can be up to 95% effective in killing adults for more than 28 days, reinfestations after this period can occur (Shipstone and Mason 1995). Most of these can be attributed to non-compliance with application instructions. Resistance of flea populations to several chemical agents has been shown to occur, including permethrin (Bossard et. al. 2002, Lemke et al. 1989), pyrethroid, and organo-phosphates (Bossard et. al. 1998).

The adverse effects of chemical control have driven the need to develop efficient flea-traps that can supplement, if not match, the effectiveness of chemical controls on the market. Since fleas use both thermal and visual cues (Müller et al 2011) for targeting hosts, most traps available on the market use incandescent light bulbs for attracting stimuli (unpublished observations). Following a thorough investigation by Dryden and Broce (1993), it was discovered that the optimal light wavelength to attract *C. felis* was in the range of 510-550 nm), and that periodic interruption of the light source dramatically increased the trapping efficiency. This feature led to comparative trials testing of several commercially available flea traps (Müller et al., 2011), including “myFleaTrap,” and “Flea Catcher.” Both use intermittent light in the correct wavelength. The current study is a comparative study of five commercially available traps in heavily cat flea infested environment in Mali, West Africa.

MATERIALS AND METHODS

Traps were delivered in their original packages. Four of them originated from China (instructions in Chinese), and one from Europe with an English user’s manual. The traps tested and their attractive features are listed below (some names may differ from the original Chinese). For an overview, see figure 1, table 1.

Figure 1: The five Flea Traps in operation mode during night time and an overview to see relative size of the tested traps.

Table 1. Overview of traps used for this study.

Trap name	Light source	# of lights	Moving/non-moving	Additional features (if any)
Flea Catcher	510 nm Green LEDs	6	non	Lights blink itermittently; larger than My Flea Tra
My Flea Trap	525 nm Green LEDs	2	non	Lights blink itermittently
Bulb Trap	White LED		non	Strong Light; No other apparent features
Biomimicry FT Trap	White and Blue LEDs	4 white; 1 blue pendulum	moving	No other apparent features
JinXin Trap	White LED		non	No other apparent features

*All traps utilize a sticky glue-board to capture attracted fleas

Trap descriptions

1: Flea Catcher. This trap can be opened in laptop like mode. It is equipped with a glue board protecting grid, three green LEDs independently oriented down and to the left, down and towards the middle and down and to the right (covering a total an angle of almost 180 degrees). Three independent light panels at the backside of the trap with different pet shapes illuminated by green LEDs (510 nm). In operation mode, the upper LEDs are synchronized with the corresponding backside panels (in pairs: left, center right), each pair is illuminated for several minutes before off time of 5 seconds followed by illumination of the next pair to be followed again by an off period. This on-off mode is repeated during trap operating time.

2: My Flea Trap. Features are similar to the Flea Catcher but with significant differences. The Flea Catcher is considerably larger being therefore a better target for jumping fleas. Furthermore, this trap has 3 LEDs and 3 corresponding light panels versus only 2 LEDs of my Flea Trap. The Flea Catcher covers a wider angle (180 degrees) compared to my Flea Trap.

3: Bulb Trap. A simple white and strong LED light source, bulb like, mounted above a glue board without any other apparent features.

4: Biomimicry FT. Square large trap body with a glue board on the bottom, illuminated by 4 white LEDs from above. In the center is a moving pendulum illuminated by a blue LED. Attraction features consist of the light in combination with movement (ie: a moving pendulum).

5: JinXin Trap: A simple white LED light source (panel-like) mounted above a glue board without any other apparent features. This trap, while similar to the Bulb Trap, is significantly smaller.

Experimental set-up

The traps were tested in five different storage rooms (A, B, C, D, E) , of 20 m^2 each, within the same farm in rural Mali. Rooms were rotated clockwise to avoid positional bias. There were more than a dozen cats and several dogs on this farm. The animals were regularly visiting the storage rooms for sleeping and catching rodents.

The animals and rooms were infested with cat fleas (*Ctenocephalides felis*). The traps were operated separately in five of these storage units and rotated to different rooms daily to avoid positional bias. The traps were operated for ten consecutive days/nights, rotating twice through the five selected rooms resulting in ten repetitions, for 24 h. Fleas and other collected insects were counted daily and removed from the glue boards.

Statistics

Student t-tests were performed to check for statistical differences between the efficiencies of the five traps while One Way Analysis of Variance (ANOVA) was used to rank significance. All statistical tests were performed with GraphPad Prism 9.00 for windows (GraphPad Software, La Jolla California, USA).

RESULTS

The two white LED "only" traps (JinXin and Bulb traps) performed very poorly at catching fleas. At the same time, they attracted large numbers of other flying insects resulting in covered, useless glue boards within one or two days (not shown). The Biomimicry FT performed slightly better at catching fleas (Figure 2) but still caught far more other non-target insects resulting in covered glue boards within 3 or 4 days. My Flea Trap caught, on average, 7.2 times more fleas than the Biomimicry trap with few non target insects; the glue board was less than 10% covered with fleas and other small insects, (mainly beetles) after the 10 days study.

The best performing flea trap was the Flea Catcher (Figure 2), outperforming the Biomimicry trap 12.3 times. Compared to my Flea Trap, its flea catches were approximately 70% higher. During the trial, fleas were observed to jump into the trap as soon as light was switched off. The presumed mechanism of action of the trap: the fleas were reacting to the relative darkness as a potential host passing in front of a light source. This trap is mainly catching fleas and very few other flying insects. The Flea Catcher caught also slightly more non target insects, mainly small beetles, as my Flea Trap but this was not an issue because of the larger glue board area.

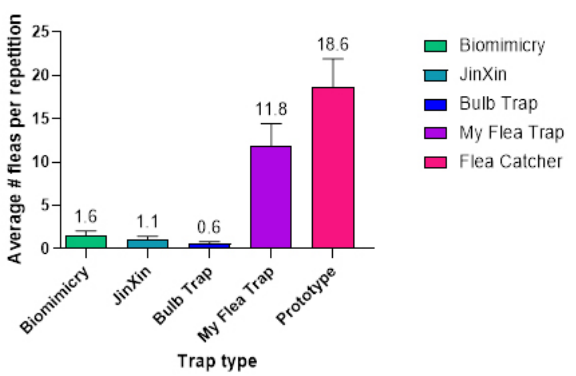

Figure 2: Observed flea catches of the 5 traps during 10 repetitions (24 hrs. operation time).

Table 2. One-way ANOVA statistical comparison amongst several varieties of flea trap.

Trap Name	Mean Diff.	Adjusted P Value
Biomimicry vs. JinXin	0.5	0.9768
Biomimicry vs. Bulb Trap	1	0.7693
Biomimicry vs. My Flea Trap	-10.2	<0.0001
Biomimicry vs. Flea Catcher	-17	<0.0001
JinXin vs. Bulb Trap	0.5	0.9768
JinXin vs. My Flea Trap	-10.7	<0.0001
JinXin vs. Flea Catcher	-17.5	<0.0001
Bulb Trap vs. My Flea Trap	-11.2	<0.0001
Bulb Trap vs. Flea Catcher	-18	<0.0001
My Flea Trap vs. Flea Catcher	-6.8	<0.0001
****Highly significant		
ns - not significant		

DISCUSSION

With the demand for alternatives to chemical pest control, increasing numbers of flea traps have come to the market. There have been few peer reviewed publications (Müller et al. 2011) confirming the efficacy of flea traps. In this study, we tested five commercially available traps for their ability to attract and catch the common cat flea. The Flea Catcher and my Flea Trap by far outperformed all other tested flea traps. This may be because of the contrast of light pulses the traps use to mimic a host passing by (Rust and Dryden 1997; Kramer and Menke 2012) or to the wavelength of light to which the fleas are attracted (Müller et al 2011). In 1993, Dryden and Broce developed a trap which had a yellow-green light (515 nm) and a 10 min-5 sec on-off cycle, and this trap was able to 86% clear a 10 m^2 room of *C. felis* in 20 h.

Another important point is that the two traps were mainly attracting fleas and not large amounts of other insects, such as moths, clogging the glue board. Interestingly, the smaller attracted storage beetles looked very much like fleas which can give consumers the impression of higher efficacy.

Also noted is that the Flea Catcher significantly outperformed my Flea Trap (Table 2). Some possible reasons for this are: the Flea Catcher has pulsing light as well as large, illuminated panels creating color contrast, and catching the attracted fleas on a much larger glue board base. Interestingly, the best performing traps were the ones with a green-yellow light source and the best trap had this light blinking for 10 min on 5 sec off in intervals (Dryden and Broce., 1993)

The label claims of the XinJin trap, the Bulb Trap, and the Biomimicry traps, that they could effectively catch fleas, could not be confirmed in this study. On the contrary these traps attracted large numbers of non-target insects including moths which clogged the complete glue boards within a few days.

In conclusion, there is wide variety in the effectiveness of flea traps on the market, all of which claim to be effective at catching these pests. The traps in this study, which half-heartedly followed scientific research into attractive features, caught 10X+ fewer fleas and many more non-targets. On the other hand, the Flea Catcher be considered user friendly.

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IN-HOUSE TESTING OF MOSQUITO POOLS FOR WEST NILE VIRUS USING COMMERCIALLY AVAILABLE IMMUNOASSAY AND REAL-TIME REVERSE-TRANSCRIPTASE POLYMERASE CHAIN REACTION KITS

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ABSTRACT

Arbovirus surveillance methods are an integral part of integrated mosquito management programs, providing information on arboviral presence, location, and transmission potential. For many vector control agencies, surveillance entails collecting vector mosquito species from the field and testing representative mosquito pools using commercially available immunoassays or real-time reverse transcriptase polymerase chain reaction test kits. In 2016, the Collier Mosquito Control District established an in-house arbovirus surveillance program to screen mosquitoes for a variety of diseases, including the endemic arbovirus, West Nile virus (WNV). Although guidance on interpreting test results is provided by the manufacturer, end users of commercial test kits are encouraged to establish their own cut-off values signifying an arbovirus positive mosquito pool. Here we report the Collier Mosquito Control District's efforts to develop cut-off values for mosquito pools using two commercially available WNV test kits.

Key Words: West Nile virus, arbovirus, RT-PCR, immunoassay

INTRODUCTION

West Nile virus (WNV; family Flaviviridae, subfamily Flavivirus) was first introduced to the State of Florida in 2001 (Blackmore et al. 2003) and continues to be a high priority disease for vector control agencies. In 2020-2021, Collier County (FL, USA) experienced heightened activity for WNV, with 11 human cases and one equine case (FLDOH, 2022). A spatiotemporal understanding of WNV vector mosquito abundance and activity is paramount to integrate mosquito management approaches to reduce WNV risk in animal and human populations. Complementary activities include arbovirus monitoring through sentinel chicken surveillance programs and/or testing of mosquito pools for arbovirus infection, both of which serve as indicators of arbovirus presence, spatial distribution, and transmission risk.

Vector-borne disease surveillance is often contracted to state public health laboratories; however, turnaround times of 2 weeks or more are standard, causing costly delays in operational responses. Thus, many vector control agencies have implemented in-house arbovirus testing programs to screen sentinel chicken sera (Peper 2021) and/or mosquito pools for arboviruses of interest. The Collier Mosquito Control District (the District) regularly tests mosquito pools from routine trap collections of Culex nigripalpus Theobald and Cx. quinquefasciatus Say from CDC miniature light traps (John W. Hock Company, Gainesville, Florida, USA), BG Sentinel traps (Biogents AG, Regensburg, Germany) and Reiter-Cummings modified gravid traps (BioQuip Products, Rancho Dominquez, California, USA) for WNV testing. Trap collections are retrieved from field sites, brought back the the District laboratory, and immediately euthanized at -80 C for 1 hr before mosquitoes are identified by morphology and pooled for arbovirus testing. Two commercially-available assays are used: the Rapid Analyte Measurement Platform (RAMP) test (Response Biomedical Corp., Burnaby, British Columbia, Canada) and the Vector Smart™ North American East (NAM-e) kit (Co-Diagnostics Inc, Salt Lake City, UT, USA). Results obtained from these tests inform operational decision making, allowing the District to respond to mosquito-borne disease threats in a timely manner.

Often, when a vector control agency identifies positive mosquito pools in-house, the pools are sent to state laboratories for secondary confirmation testing. This is of particular importance in Florida as positive pools are not included on the Florida Department of Health arbovirus report unless they receive secondary confirmation by the Bureau of Public Health Laboratories (Tampa). The District has observed that samples testing positive for WNV using in-house methods do not always test positive when sent to the state laboratory for confirmation, an observation previously reported by Burkhalter et al. (2014) for mosquito pools that had initially tested positive using the RAMP test. Here we report the District's efforts to establish operationally relevant cut-off values for mosquito pools testing positive for WNV when using RAMP test and the Vector Smart NAM-e kit. Determining these cut-off values increases the probability that secondary confirmation testing will be successful and provides a baseline for when a vector control agency should take operational action in response to positive pool results.

The RAMP test is a common immunoassay utilized by vector control agencies to detect WNV antigen in pools of local mosquitoes. Pools of up to 50 female mosquitoes are homogenized in a manufacturer-supplied RAMP buffer. An aliquot of the processed homogenate is combined with fluorescently-bound WNV antibodies, which bind to WNV if it's present in the sample. The homogenate-antibody mixture is transferred to the RAMP WNV test cartridge. The mixture migrates along a test strip through capillary action, and WNV-bound antibodies become immobilized at the detection zone. After a 90-minute incubation period, the test cartridge is inserted into a RAMP reader, which provides a fluorescence reading ranging from 10.0 to 640.0 RAMP units.

The manufacturer indicates that readings of ⩾ 30 RAMP units as the cut-off for positive WNV mosquito pools but encourages end users to set their own local cut-off values and establish "gray zones" of uncertainty (Response Biomedical 2016). Although several agencies have published their own cut-off values and interpretation guidelines (Burkhalter et al. 2006, Williges et al. 2009, Kesavaraju et al. 2012, Burkhalter et al. 2014, Coatsworth et al. 2022), the Centers for Disease Control and Prevention (CDC) suggests using a cut-off value of 50 RAMP units for positive WNV mosquito pools that do not require additional confirmatory testing (Buckhalter et al. 2014, Response Biomedical 2016). For tests that do require secondary confirmation, the CDC suggests a more conservative approach, defining a "gray zone" for readings between 50-100 RAMP units that are likely to contain some amount of virus but may not be able to be confirmed via real time reverse transcriptase polymerase chain reaction (qRT-PCR) due to the inhibitory action of the RAMP buffer (Burkhalter et al. 2014, Response Biomedical 2016).

The Vector Smart NAM-e kit is a qRT-PCR based multiplex assay that has recently become available to vector control agencies for mosquito pool testing of WNV, Saint Louis encephalitis virus, and eastern equine encephalitis virus. The District was an early adopter of the test kit and has used it as the primary mosquito pool test method since 2019. In this assay, mosquito pools of approximately 25 females are homogenized in 1x phosphate buffer saline. Total nucleic acid is extracted using the MagMAX CORE Nucleic Acid Purification kit (ThermoFisher Scientific, Waltham, MA, USA) in the KingFisher™ Duo Prime Purification System (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Total nucleic acid is then used with the Vector Smart NAM-e kit, and arbovirus detection data is generated on Applied Biosystems® QuantStudio® 5 Real-Time PCR System (Thermofisher, Carlsbad, CA) following manufacturer guidelines, which includes the use of an internal, positive and negative control. The manufacturer recommends that positive cut-off cycle threshold (Ct) values be determined through in-house validation testing.

Although WNV is endemic in Collier County, from 2017-2019 mosquito infection rates were rarely high enough to be detected by the District's arbovirus surveillance program using RAMP assay or qRT-PCR methods. Further, WNV human and equine infections were low during the same timeframe, with only one WNV equine infection reported in 2017 (FLDOH 2022). However, an unusually large number of mosquito pools tested positive for WNV in 2020 and 2021. Due to staff limitations, supply chain delays, and laboratory accessibility constraints associated with the COVID-19 pandemic, the District used either RAMP or Vector Smart NAM-e kits for testing during this period. A total of 2,286 pools were tested, 32 of 579 tested by using RAMP (cut-off: ⩾ 30 RAMP units) were positive, and 23 of 1,707 tested using the Vector Smart NAM-e kit (cut-off: Ct value ⩽ 40) were positive. Processed homogenate (in PBS or RAMP buffer) of positive pools were sent to the state laboratory for confirmation testing.

Of the RAMP positive pools, 21 were sent to the state laboratory for confirmation testing. Five had readings over 100 RAMP units, six fell within the gray zone (50-100 RAMP units) defined by Burkhalter et al. (2014) and the remainder had readings of less than 50 RAMP units but exceeded the manufacturer's recommended cut-off of 30 RAMP units (Table 1). Only three of the 21 pools were confirmed positive using RT-PCR (FLDOH, 2022), and the readings for all three exceeded 130 RAMP units. Based on a conservative interpretation of these results, the District has defined readings between 30-100 RAMP units as our gray zone and categorizes pools with readings falling in this range as "marginally positive," with the expectation that these pools are unlikely to test positive if sent for RT-PCR based confirmation testing (Burkhalter et al. 2014,

Table 1: Mosquito pools tested in-house via RAMP assay. Trap types include: CDC miniture light traps (CDC), BG-Sentinel traps (BGS) and and Reiter-Cummings modified gravid trap (GRV). Red highlight signifies confirmed positive samples, grey highlight signifies mariginally positive samples, and green highlight signifies negative samples.

Collection Date	Trap Type	Species	Number Mosquitoes	RAMP Units	Confirmation
9/1/20	CDC	Cx. nigripalpus	25	640	Detected
9/15/20	CDC	Cx. nigripalpus	25	269.9	Detected
9/15/20	CDC	Cx. nigripalpus	25	133.1	Detected
10/14/20	CDC	Cx. nigripalpus	25	116.2	Not detected
9/1/20	CDC	Cx. nigripalpus	25	112.1	Not detected
9/25/20	CDC	Cx. nigripalpus	25	98.5	Not detected
10/6/20	CDC	Cx. nigripalpus	25	84.5	Not detected
9/15/20	CDC	Cx. nigripalpus	25	79.2	Not detected
9/20/20	CDC	Cx. nigripalpus	25	71.9	Not detected
10/6/20	CDC	Cx. nigripalpus	25	60	Not detected
9/18/20	CDC	Cx. nigripalpus	25	53.9	Not detected
11/17/20	CDC	Cx. nigripalpus	25	*53.5*	*Not submitted*
11/9/21	CDC	Cx. nigripalpus	25	*48.3*	*Not submitted*
7/27/21	CDC	Cx. nigripalpus	25	*46.5*	*Not submitted*
10/6/20	CDC	Cx. nigripalpus	25	46.1	Not detected
12/4/20	CDC	Cx. nigripalpus	19	*43.5*	*Not submitted*
12/14/20	CDC	Cx. nigripalpus	25	*42.4*	*Not submitted*
10/6/20	CDC	Cx. nigripalpus	25	39.3	Not detected
10/8/20	GRV	Cx. quinquefasciatus	1	39.2	Not detected
11/17/20	CDC	Cx. nigripalpus	25	*36.7*	*Not submitted*
2/17/20	CDC	Cx. nigripalpus	25	*35.8*	*Not submitted*
2/17/20	CDC	Cx. nigripalpus	25	*35.8*	*Not submitted*
9/23/20	CDC	Cx. nigripalpus	25	35.6	Not detected
10/6/20	CDC	Cx. nigripalpus	25	32.2	Not detected
9/1/20	CDC	Cx. nigripalpus	25	32.1	Not detected
10/6/20	BGS	Cx. nigripalpus	21	31.8	Not detected
7/27/21	CDC	Cx. nigripalpus	25	*31.5*	*Not submitted*
10/6/20	CDC	Cx. nigripalpus	25	31.3	Not detected
7/16/21	GRV	Cx. nigripalpus	4	31	Not detected
11/17/20	CDC	Cx. nigripalpus	25	*30.6*	*Not submitted*
11/2/21	BGS	Cx. nigripalpus	25	*30.6*	*Not submitted*
10/8/20	BGS	Cx. nigripalpus	2	30	Not detected

Response Biomedical 2016). Marginally positive mosquito pools are taken into consideration by the District when making operational decisions but do not fully dictate treatment decisions. Pools with readings exceeding 100 RAMP units are considered positive for WNV, sent to the state laboratory for testing, and used to make operational treatment decisions.

Of the pools that tested positive using the Vector Smart NAM-e kit, 22 were sent to the state laboratory for confirmation testing. Eight of these were confirmed positive using the state's method of RT-PCR, and an additional three were considered "equivocal" (FLDOH, 2022). Equivocal results were due to the RT-PCR detecting WNV using one primer set but not the other (A. Morrison, personal communication; August 10, 2021). There was some overlap in the Ct values for the samples that were confirmed at the state level; confirmed samples had Ct values ranging from 21-36, and unconfirmed samples had

Table 2: Mosquito pools tested in-house via Vector Smart NAM-e kit. Trap types include: CDC miniture light traps (CDC), BG-Sentinel traps (BGS) and and Reiter-Cummings modified gravid trap (GRV). Red highlight signifies confirmed positive samples, grey highlight signifies mariginally positive samples, and green highlight signifies negative samples.

Collection Date	Trap Type	Species	Number Mosquitoes	Ct Value	Confirmation
8/24/21	BGS	Cx. nigripalpus	25	20.9	Detected
8/27/21	GRV	Cx. quinquefasciatus	14	21.4	Detected
8/20/21	GRV	Cx. quinquefasciatus	25	22.2	Detected
8/6/21	GRV	Cx. quinquefasciatus	4	24.3	Detected
8/5/20	BGS	Cx. nigripalpus	17	24.7	Detected
8/20/21	GRV	Cx. quinquefasciatus	25	27.5	Detected
7/13/21	CDC	Cx. nigripalpus	25	28.3	Detected*
7/13/21	CDC	Cx. nigripalpus	25	29.3	Equivocal*
7/13/21	CDC	Cx. nigripalpus	25	30.7	Equivocal*
7/13/21	CDC	Cx. nigripalpus	25	33.9	Equivocal*
7/13/21	CDC	Cx. nigripalpus	25	34.2	Not detected*
8/24/21	BGS	Cx. nigripalpus	25	34.4	Not detected
8/5/20	BGS	Cx. nigripalpus	25	36.2	Not detected
6/22/21	CDC	Cx. nigripalpus	16	36.4	Not detected
8/10/21	CDC	Cx. nigripalpus	25	36.4	Not detected
8/27/21	GRV	Cx. nigripalpus	11	36.5	Detected
6/25/21	GRV	Cx. quinquefasciatus	25	37.5	Not detected
7/13/21	CDC	Cx. nigripalpus	25	37.8	Not detected*
7/28/21	GRV	Cx. nigripalpus	14	38.4	*Not submitted*
8/20/21	GRV	Cx. nigripalpus	1	38.5	Not detected
8/27/21	GRV	Cx. quinquefasciatus	10	38.7	Not detected
8/24/21	GRV	Cx. nigripalpus	25	38.9	Not detected
8/10/21	BGS	Cx. nigripalpus	17	39.2	Not detected

* Shipping issues may have had impact on confirmatory testing

values ranging from 29-39 (Table 2). Due to shipping issues, pools with a collection date of July 13, 2021 are noted in Table 2; however, WNV positive homogenate has been shown to be stable at ambient temperatures (Erandio et al. 2020). Based on these results, the District has defined samples with Ct values between 31-37 as marginally positive and pools with Ct values ≤ 30 as positive. As with the RAMP test, marginally positive mosquito pools are considered operationally but do not dictate actions, while positive mosquito pools strongly influence treatment decisions. All pools testing either marginally positive or positive using the Vector Smart NAM-e kit are sent to the state laboratory for testing.

As of 2017, almost all independent mosquito control agencies in Florida reported in-house arbovirus testing of mosquito pools, whereas more than half of dependent mosquito control agencies depended exclusively on state-level testing (Moise et al. 2020). The District typically has a 24-48 hr turnaround time between when mosquitoes are captured and when pool results are available to operational decisionmakers, while state testing takes 1-2 weeks at a minimum. In-house testing therefore minimizes the delay between sample acquisition and operational decision-making and represents a significant improvement in the District's ability to respond quickly to emergent disease threats. The cut-off values outlined here are appropriate for District uses, but variations in the arbovirus being tested, mosquito species, local populations, and laboratory protocols can influence pathogen detection measurements (Kesavaraju et al. 2012, Burkhalter et al. 2014, Response Biomedical 2016). For this reason, agencies should develop cut-off values tailored to their particular testing conditions in order to increase confidence in arboviral test results and help optimize organizational responses to mosquito-borne disease threats.

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OPERATIONAL NOTE

AN ASSESSMENT OF A LETHAL OVIPOSITION TRAP'S ABILITY TO PRODUCE IMMATURES OF AEDES ALBOPICTUS IN PANAMA CITY BEACH, FLORIDA

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ABSTRACT

A series of tests were performed in the laboratory and in the field, 2014-2017, assessing the efficacy of the Trap 'N' Kill (TNK) (Springstar® Inc., Woodinville, WA) lethal ovitrap on *Aedes albopictus* adults and immatures. The TNK lethal ovitrap design is to attract and kill adult female mosquitoes as well as any larvae that emerge over a claimed 45-day period by utilizing the active ingredient dichlorvos. Cage trials demonstrate that the trap effectively killed wild-caught, adult mosquitoes (> 80% mortality) for up to 4 weeks (28 days). After 4 weeks, the ability of the trap to control adult mosquitoes declined reaching only 18.8 % mortality by week 7. A field trial demonstrated a similar 4-week maximum effective duration of the TNK trap when immature mosquitoes had emerged from eggs and were present in all traps after 4 weeks (6 weeks, 15.2 ± 2.56 % larvae per trap were observed). A field trial was also conducted to assess the ability of the TNK trap to reduce adult mosquito populations over a ~5-acre area in a residential neighborhood. No reduction in adult mosquito population could be detected with the trap density (5 traps/acre) and experimental conditions utilized in this trial.

Key words: *Stegomyia albopicta*, Culicidae, lethal ovitrap, dichlorvos, oviposition

INTRODUCTION

Aedes albopictus (Skuse) is an invasive container inhabiting mosquito species that is considered competent in the transmission of many pathogens including dengue, chikungunya, and zika viruses (McKenzie et al., 2019). *Aedes albopictus* is a diurnal ovipositor and an exophilic mosquito and it is considered a social nuisance due to temporal feeding patterns that are associated with humans. *Aedes albopictus* also demonstrates skip ovipositional behavior which enhances its ability to compete for larval habitat, and therefore, its distribution and success in domesticated habitats (Xue and Barnard 1997, Trexler et al. 1997, Richards et al. 2006, Davis et al. 2015). Human association with *Ae. albopictus* can cause concern for public health mosquito control operations due to 1) anthropogenic adaptations, 2) being an efficient vector, 3) difficult to survey with traditional trapping methods and, 4) difficult to control due to her diurnal feeding behavior (Hoel et al. 2009, Unlu et al. 2014, Ayllon et al. 2018). Artificial containers (tires, buckets, play toys, boats, discarded tarps, etcetera.) regularly show abundant immature populations of *Ae. albopictus* in peri-domestic habitats (Hawley et al. 1988, Sota and Mogi 1992, Simard 2005). The preference for artificial containers as oviposition habitats demonstrated by *Ae. albopictus* can be exploited for the surveillance or control in the form of lethal oviposition traps (Perich et al. 2003, Zeichner 2011, Buckner et al. 2017).

Little black jars (LBJ) are the standard for measuring the presence/absence of container-inhabiting mosquitoes (Conner and Monroe 1923, Soper 1965, Fay and Eliason, 1966), whereas the color of the trap can assist in attracting and influencing oviposition (Hoel et al. 2011, Dixson et al. 2020). The Trap 'N' Kill (TNK) (Springstar® Inc., Woodinville, WA) is a lethal ovitrap designed to attract and kill adult female mosquitoes who enter the trap to oviposit as well as larvae that emerge from eggs oviposited. Improvements to lethal ovitraps with the introduction of the TNK trap by adhering "substrate tabs" with pesticide residues, which can achieve a level of lethality toward container inhabiting *Aedes*, enabling control within the ovipositional habitat (Perich et al. 2003, Zeichner 2011). The trap is black plastic and consists of a source of water to attract gravid female mosquitoes, a red velour strip as an oviposition substrate and a dichlorvos embedded tab, stapled to the interior wall of the trap. The TNK is designed to kill adults and immatures for as long as the dichlorvos embedded strip is systematically replaced. The manufacturer recommends replacement of the dichlorvos strip after 45 days to maintain the trap's effectiveness.

A series of experiments were conducted in 2014 to assess the efficacy of the dichlorvos tab in the TNK lethal ovitrap due to its availability and accessibility to homeowners as well as the manufacture's claim of 45-days of control. A caged trial was performed in Panama City Beach, FL at Beach Mosquito Control District's laboratory for 7 weeks to evaluate the length of time the active ingredient dichlorvos would cause mortality among wild caught female adult *Ae. albopictus.* Fifty gravid mosquitoes were placed in 6 cages every 7 days for 7 weeks. The results indicated that mortality remained above 80% for 4 weeks. After 4 weeks the average percent mortality dropped significantly (Figure 1) until reaching 18.8% by week 7. This result strongly suggests that the dichlorvos embedded strip begins to lose effectiveness after 30 days.

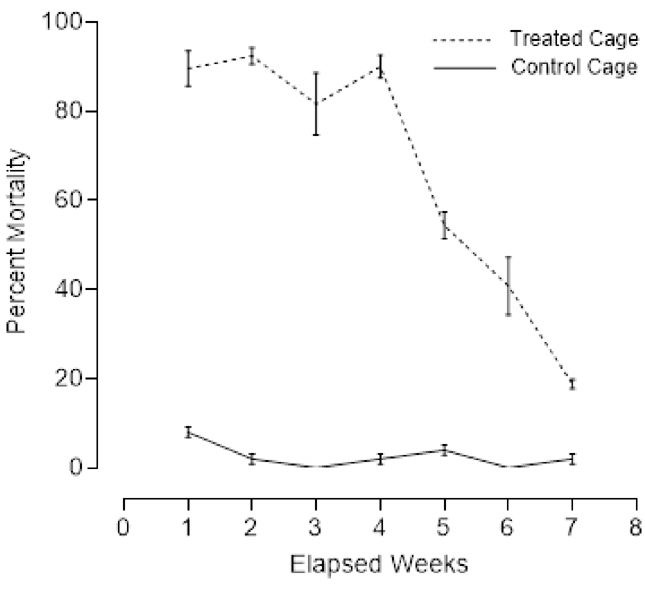

Figure 1. Descriptive trends indicating 4th-week interval percent mortality drop significantly, whereas the standard error of the mean is applied to the control observation as well as the treatments.

In Panama City Beach, FL a field trial was conducted with 5 TNK traps at a site (30°13'31.2"N 85°52'54.0"W) known for *Ae. albopictus* activity (n=498, Adult (F), 2014-2017) for 6 weeks. In all 5 traps larval mosquitoes were observed after the 4th week interval in the presence of the pesticide tab eventually reaching 15.2 ± 2.56 % larvae per trap in week 6 (Figure 2).

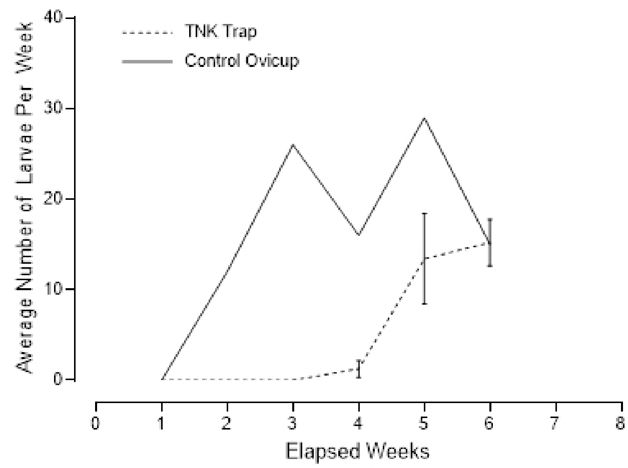

Figure 2. The average number of larvae present per week, where 4th week into the assessment larvae were present in the presence of the pesticide.

All immatures were identified (Darsie and Ward, 2005) as *Ae. albopictus* 89%, *Ae. japonicus* 8% and *Ae. triseriatus* 3%. For these results, we concluded that a 30-day maintenance interval would be superior to the recommended 45 days interval due to the counterproductive observation of larval mosquitoes in the lethal ovipositional environment.

In 2017, a 12-week (<90 consecutive trapping days) trial was conducted, in a dense residential neighborhood (30°10'36.1"N 85°46'49.0"W) backed up against a major drainage ditch flowing southward and placed to the east of the site. This site known for *Ae. albopictus* activity (n=1,526 female adults and n=10,000 larvae collected, 2015-2016) was selected for the deployment of 24 TNK traps at a density of approximately 5 traps per acre. A control site was selected (30°09'06.0"N 85°45'36.2"W) based on common habitat and activity of *Ae. albopictus* (n= 1,321 female adults and 389 larvae collected, 2015-2016) where 24 non-treated LBJ's were arranged. TNK trap sites were selected to prevent any possible bordering influences. The adult populations of *Ae.* albopictus were sampled once per week for a 24-hour period (<12 trapping days) in both the treated and controlled areas with 12 BG-Sentinel 2 (BG2) traps (Biogents AG, Regensberg, Germany) divided equally amongst both sites. Bio Gents 2 traps utilized the attractant BG Lure (Biogents AG, Regensberg, Germany) only; carbon dioxide was not used during this study. Due to battery failures, trap tampering or other confounding factors, not every location/treatment pair yielded the full 6 mosquito samples for each sampling date. The number of female *Ae. albopictus* for each trap location/observation date/treatment combination was averaged (mean) and a standard error was calculated to represent average mosquitoes per day (24 hours). To enable a statistical comparison between observation dates within each treatment as well as enable a comparison between the treatment and control, a one-way ANOVA was selected. There was no significant reduction (p= 0.22; one-way ANOVA) of adult populations (n=1,592 adult females) in the treatment area on any of the days sampled by the BG2 trap (Figure 3). There were also no significant differences in average female mosquitoes between observation dates within each treatment. No adult mosquito control applications were conducted during this study.

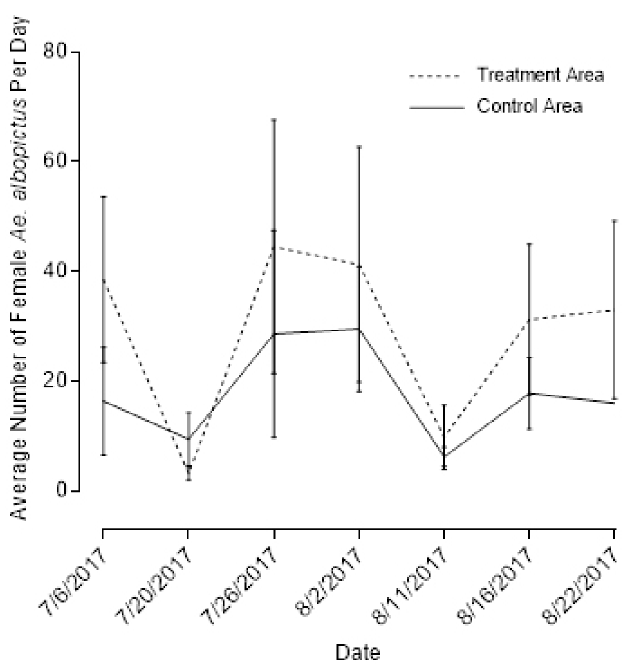

Figure 3. The population of Aedes albopictus was sampled by BG sentinel traps in both the treated and control areas. There was no significant reduction (P=0.22; One-Way ANOVA) in the treatment area or between sampling dates on any of the days sampled.

Each TNK trap was inspected once per week. After 30 days pesticide tabs, 300 mL water, and ovipostion substrates were replaced. Egg deposits were counted and identified as hatched (12%) or unhatched (88%). A portion of the eggs in the treatment site were observed to have hatched (n=586 hatched eggs; n= 4943 unhatched eggs) with no observation of any immature *Ae. albopictus* (n=0 larvae) inside the TNK trap during the 30-day interval. Using 30-day intervals for maintenance, zero larval mosquitoes were observed during the 12-week trial in the treatment area. The control site larvae of *Ae. albopictus* were present in sampling (n=296 larvae; n=2367 eggs), then identified to 4th instar larvae (Darsie and Ward, 2005) and preserved in 80% ethanol. *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) was not observed during this study.

These experiments demonstrated a few important conclusions about the TNK lethal ovitrap. Firstly, a 45-day replacement schedule is inadequate to prevent the development of larval mosquitoes within the trap. A 30-day replacement schedule would ensure no development of larval mosquitoes and greater than 80% mortality of adults that enter the trap. The manufacturer's website (https://www.springstar.net) recommends up to a 10-week replacement interval (the product instructions do state only 45 days). From our observations, either interval (45 days or 10 weeks) would be completely inadequate in maintaining the efficacy of this trap. Secondly, this study demonstrates the critical importance of regular maintenance to prevent the trap from becoming additional mosquito habitat. After 4 weeks, all traps in our study contained larval mosquitoes of all instars. Taken together with the observation that mortality of adult mosquitoes declines dramatically after four weeks, it is likely this trap can produce adult mosquitoes if not serviced within a 30-day period. The manufacturer recommends up to 30 traps per acre to control mosquitoes in the area. This number of traps, if not properly maintained, have the potential to become a significant source of larval habitat. Thirdly, this study evaluated the effect the TNK trap might have on natural adult mosquito populations at a manageable density of ~5 traps per acre (24 traps in total). This density is below the manufacturer's recommendation but represents a manageable number of traps for a homeowner. At this density, no effect on adult mosquito populations could be discerned when assessed by the BG Sentinel 2. The manufacture does recommend a much higher trap density to be effective (up to 30 traps per acre). Individual residential lots at this site in Panama City Beach, FL are up to 0.5 acres, accommodating the manufactures suggestions for control 15 traps would need to be placed per lot. Each trap would cost the homeowner $11.00-18.00

US at total cost of $165.00-270.00 US for a total of 3-month interval for control with the supplies included. We found the monthly maintenance of only 24 traps to be laborious and the level of attention and maintenance required to prevent the trap becoming additional larval habitat may present a substantial impediment for the average homeowner. To utilize anywhere near the recommended number of traps in our 5-acre study site (~150 traps) we suggest would be impracticable. In conclusion, the TNK trap is effective in killing adult and larval mosquitoes but for a much shorter interval than advertised. In addition, the dependence of this trap on regular maintenance and the high trap density required to affect mosquito populations suggests that it would be best utilized for small areas that would enable a limited number of traps to be serviced regularly.

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OPERATIONAL NOTE

FIELD EVALUATION OF AUTOCIDAL GRAVID OVITRAP AND SIRENIX TRAP AGAINST CONTAINER INHABITING MOSQUITOES IN SAINT AUGUSTINE, NORTHEASTERN FLORIDA

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ABSTRACT

Mosquito control programs are utilizing cost-effective long-term autocidal traps targeting the gravid population of container-inhabiting and other mosquito species, with the aim of reducing vector populations and disease transmission risk. In this field study we directly compared the efficacy of two autocidal trap types-the Autocidal Gravid Ovitrap (AGO) and SIRENIX mosquito trap in reducing mosquito abundances in St. Augustine, Florida to a control only site that had no autocidal traps deployed. Pre-treatment (wk1-4) and post-treatment (wk 5-14) adult mosquitoes were captured in all three sites using BG traps baited with BG lure and dry ice. Pre- and post-treatment trap counts of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, and total mosquitoes (three species together) were compared to determine significant changes in abundance. Percent reduction in abundance of each species/group at the two trap sites were calculated to evaluate the trap efficacy at controlling *Aedes* and *Culex* container mosquitoes. *Aedes albopictus* populations were significantly reduced (86.6%) at the SIRENIX site compared to the populations at the AGO site (67.7% reduction). *Ae. aegypti* populations were reduced by 72.4% at the SIRENIX site compared to 25% at the AGO site. *Culex quinquefasciatus* population reduction at the SIRENIX site was 59.6% compared to 11.8% at the AGO site. The total mosquito group had only 45.1% and 10.3% reduction at the SIRENIX and AGO sites, respectively. Furthers studies conducted across the entire mosquito season would be required for full understanding of the effectiveness of these traps.

Key Words: *Aedes aegypti*, *Aedes albopictus*, Autocidal Gravid Ovitraps, Sirenix traps, population reduction

INTRODUCTION

Container inhabiting mosquitoes, *Aedes aegypti* (Linn).) and *Aedes albopictus* (Skuse), are selective domestic species that mostly oviposit in natural and man-made water containers associated with human dwellings and activities. This association with domestic dwellings makes control of these important nuisance and vector species difficult. Not only due to human behavior, including water storage practices, but gaining access to domestic areas for operational mosquito control technicians for prevention can be restricted and even prevented, allowing for these populations to become unmanageable. Thus, there is a need to develop tools allowing for better domestic mosquito control.

Mosquito oviposition behavior (Bentley & Day 1989) has been a main target to develop novel approaches and tools for mosquito surveillance and monitoring vector population dynamics, and vector control of highly domestic mosquito species (Reiter, 1983, Chadee and Corbet, 1987, Eiras et al. 2014). The first trap device used a combination of mechanical suction and organic plant-based infusion to collect eggs and attract gravid females (Reiter, 1983). Oviposition traps lined with polybutylene adhesive were successful to collect both *Ae. aegypti* and *Culex quinquefasciatus* Say in Australia (Barbosa et al., 2010). This approach was further exploited and developed in attract-and-kill ovitraps and gravid traps, with the added advantage of attracting older mosquito cohorts that might be actively involved in disease transmission (Day, 2016). Some field trials have been carried out to compare the efficacy of different trap types, such as gravid traps and Autocidal Gravid Ovitraps (AGOs) under urban environmental conditions (Cilek et al. 2017) and AGOs and In2Care traps (Buckner et al. 2017, Autry et al. 2021, Khater et al. 2022), where different levels of efficacies were observed (Su et al. 2020). The purpose of this operational note is to provide information on the new SIRENIX trap available for use in mosquito control in comparison to the already established attract and kill AGO trap.

The AGO is a dual action surveillance and control tool that aims at capturing and killing gravid females of container-inhabiting *Aedes* mosquitoes (Barrera et al. 2014 a, b). The AGO trap was purchased from AP&G (Catchmaster, USA). The trap consists of a 19-L black bucket with a fitted lid that houses a removable capture chamber (Figure 1). 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 have no access to the water. Each AGO trap requires 8 L of water and no pheromones or pesticides are required. Holes were drilled at the 8-L mark to prevent excess water from rain or irrigation but small enough to avoid mosquito entry into the trap. The AGO traps were placed under trees, shrubs, and in the backyards to prevent damage. These traps were monitored weekly to add water as needed.

Figure 1. AGO trap consists of the black polyethylene pail with lid and a sticky surface coated to adhesive paper for trapping of gravid females.

The SIRENIX traps were provided by New Mountain Int'l Pte Ltd, Marathon, FL. These traps were developed with the aim to attract gravid female mosquitoes. The trap consists of an integrated acoustic source (IAS), a detachable solar panel, and a hatching basin (Figure 2). SIRENIX traps are acoustic larviciding devices, which expose mosquito larvae to acoustic energy within a certain frequency band resulting in the rupture of the walls of the dorsal tracheal trunks (DTTs), causing the expulsion of gas into the body cavity, resulting in mortality, arrested larval development, or flightless adult mosquitoes (Nyberg & Muto, 2020). The SIRENIX traps were placed

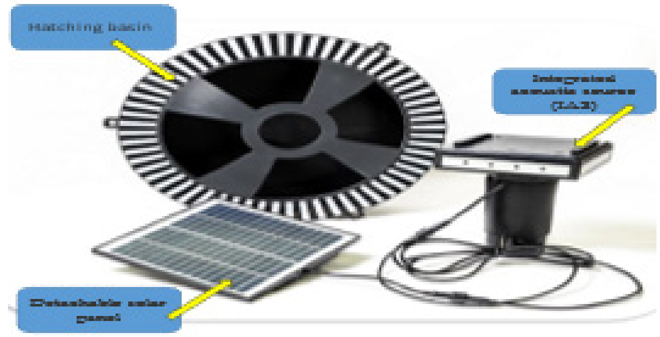

Figure 2. SIRENIX trap consists of the integrated acoustic source (IAS), detachable solar panel, and hatching basin.

under trees, shrubs, and in the backyards to prevent damage or removal. These traps were monitored weekly to add water as needed. Additionally, the solar panels that charge the traps IAS were placed in direct sunlight even when the traps were placed in the shade.

For this study, 100 AGOs and 100 SIRENIX traps were evaluated. Three sites were selected in downtown St. Augustine, Florida, based on historical data on the abundance of *Ae. aegypti* and *Ae. albopictus* mosquitoes (Smith et al. 2018). The selected sites were 18 acres (7.28 hectares) in size and 700 meters apart. Site one was treated with AGOs, site two was treated with SIRENIX traps, and the third site was selected as an un-treated control site (Figure 3). The AGO site had 38 houses and averaged 2.6 traps per house. The SIRENIX site had 42 houses and averaged 2.3 traps per house. All the traps were deployed over a one-day period, preceded by providing the residents with educational brochures of the different traps being evaluated. All 200 traps, 100 of each type were set by mosquito control professionals. The pre-treatment period was from April 21 to May 10, 2022. AGO and SIRENIX traps were deployed on May 11, 2022 and the remaining ten-week post-treatment evaluation was from May 11 to July 26, 2022. Trap efficacy was evaluated using BG traps baited with BG lure and dry ice. The AGOs and SIRENIX traps were used in the treatment period only. Three BGs were deployed per site and were set weekly throughout the 14-wk study period. Adult mosquitoes were collected from the BGs traps after 24 hr. The collected mosquitoes were transferred to the Anastasia Mosquito Control District (AMCD) lab for counting and identification of adult mosquito species.

All statistical analyses for AGO and SIRENIX trap data were analyzed using IBM®SPSS®Statistics –version 20. The pre- and post–treatment abundances of *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* and total mosquitoes (all 3 species together) were compared at the three sites using Mann_Whitney test. Weekly differences in abundances

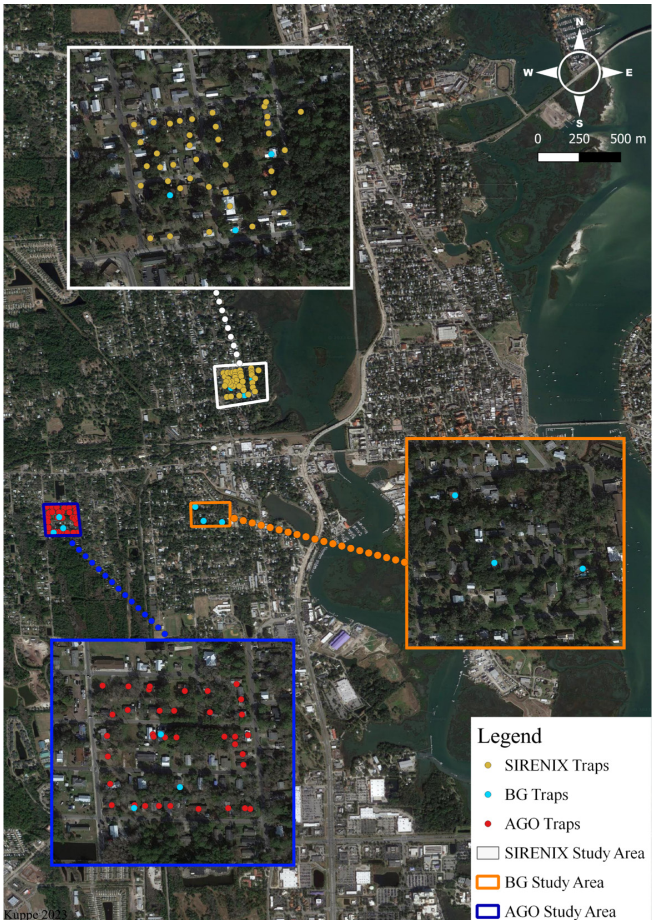

Figure 3. Location of the AGO, SIRENIX, and control sites with the different trap locations in urban areas of St. Augustine, Florida.

were compared using Kruskal Wallis test. The significance level was set to 0.05 for all comparisons. The overall mean percent reduction of each species/group was calculated using Mulla's equation (Mulla et al. 1971).

For this study, we used the collections of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* (urban container breeders) collected in the BG traps. The total number of mosquitoes processed during this 14-week study was 14,055 (combined urban container breeders)with *Cx. quinquefasciatus* representing 47.3% of the collections (n=6644). *Aedes aegypti* represented 33.8% (n=4755) of the collections with *Ae. albopictus* representing 18.9% of the collections (n=2656).

The abundances (mean ± SD) of *Ae. aegypti* and *Ae. albopictus* at all three sites were low during the pre-treatment period (Table 1). Post-treatment abundances at all three sites, except the *Ae. albopictus* abundance at the SIRENIX site was significantly higher than the pre-treatment abundances (*Ae. aegypti*: AGO U=102.5, P=0.026, SIRENIX U=79.0, P=0.005, control U=28.0, P<0.0005, and *Ae. albopictus*: AGO U=104.5, P=0.035, SIRENIX U=166.0, P=0.695, control U=73.0, P=0.002). However, according to Mulla's percent reduction, both species had achieved overall reductions at both trap sites compared to the control site. Overall percent reduction of *Ae. aegypti* at the SIRENIX site (72.4%) was almost 3 times higher than that of the AGO site (25%). Percent reduction of *Ae. albopictus* was higher at the SIRENIX site as well (86.6%) still with a high percent reduction at the AGO site (67.7%).

The important West Nile vector, *Cx. quinquefasciatus* had lower post-treatment abundances at all three sites (Table 1). However, the reduction of the post-treatment abundance was significant only at the SIRENIX site (AGO U=138.0, P=0.242; SIRENIX U=77.5, P=0.004; control U=126.0, P=0.132). SIRENIX site demonstrated 59.6% reduction in *Cx. quinquefasciatus* while the AGO site had only 11.8 % reduction compared to the control site

Post-treatment abundances of total mosquitoes were higher at both control and AGO site while it was lower at the SIRENIX site (Table 1). None of those changes were significant (AGO U=169.0, P=0.759: SIRENIX U=160.0, P=0.577: control U=150.5, P=0.411). The overall percent reduction of total mosquito abundance in the SIRENIX site was 45.1% while that in the AGO site was 10.3%.

All species/group had significant weekly changes in abundance at the control site ($X^2_{(13)}$=35.12, P=0.001, $X^2_{(13)}$=31.1, P=0.003, $X^2_{(13)}$=22.98, P=0.043, and $X^2_{(13)}$=32.58, P=0.002 respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes. Posthoc pairwise comparisons of Kruskal Wallis test demonstrated significant increases of *Ae. aegypti* and *Ae. albopictus*

Table 1. The pre- and post-treatment abundances (mean ± SD) of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, total mosquitoes (three species combined at all three sites).

	AGO		SIRENIX		Control	
	Pre	Post	Pre	Post	Pre	Post
Aedes aegypti	0.6 ± 0.7	9.9 ± 15.7	5.2 ± 3.1	33.2 ± 35.4	1.8 ± 1.3	42.0 ± 39.8
Aedes albopictus	6.1 ± 8.0	30.9 ± 36.9	4.8 ± 3.2	9.7 ± 12.0	0.3 ± 0.6	3.8 ± 5.4
Culex quinquefasciatus	51.2 ± 41.5	33.2 ± 28.2	68.3 ± 80.1	18.4 ± 20.7	56.3 ± 35.7	37.6 ± 32.7
Total mosquitoes	57.8 ± 39.8	74.1 ± 67.2	78.2 ± 79.8	61.3 ± 55.9	58.4 ± 36.2	83.4 ± 65.1

abundances from the 9th week (i.e. the 5th post-treatment week) than all the pre-treatment week abundances ($P<0.05$ for all). After the 9th week, the abundance remained high. *Culex quinquefasciatus* had significantly lower abundance in the 5th week than the 1st, 3rd and 4th weeks ($P<0.05$ for all). The differences in abundance of total mosquitoes were only between a few post-treatment weeks. However, there were no such significant weekly differences at the AGO site ($X^2_{(13)}=21.08$, $P=0.071$, $X^2_{(13)}=18.73$, $P=0.132$, $X^2_{(13)}=15.06$, $P=0.303$, and $X^2_{(13)}=14.65$, $P=0.33$ respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes) or the SIRENIX site ($X^2_{(13)}=21.01$, $P=0.073$, $X^2_{(13)}=18.39$, $P=0.143$, $X^2_{(13)}=20.89$, $P=0.075$, and $X^2_{(13)}=16.53$, $P=0.222$ respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes).

In this study, the field effectiveness of two autocidal mosquito traps, the AGO and the new SIRENIX trap, were directly compared as control tools, mainly against important container-inhabiting mosquitoes. Based on overall percent reductions, the SIRENIX trap was more effective than AGO trap in reducing container-inhabiting *Aedes* and *Culex* populations. The SIRENIX trap was more effective against *Aedes* species than *Culex*. The lower effectiveness against *Cx. quinquefasciatu*s contributed more to the total mosquito abundance. This is not surprising as *Cx. quinquefasciatus* is commonly found in other breeding sites such as storm drains, ditches, and abandoned pools, habitats that are not conducive to *Ae. albopictus* or *Ae. aegypti*, providing additional breeding sites for population proliferation. Results demonstrated that SIRENIX traps would be a good option in the field to control container inhabiting *Aedes* mosquitoes. It should be noted that these traps were deployed and then removed from the field prior to the peak of *Aedes* container-inhabiting populations and should be re-evaluated for an entire mosquito season for full understanding of the role of these traps in an integrated mosquito surveillance and control program.

In a previous study, the AGOs and In2Care traps had a significant impact on reducing adult *Ae. aegypti* populations, with the AGO traps being relatively more effective than the In2Care traps (Autry et al. 2021, Khater et al. 2022). In Puerto Rico, AGO traps reduced *Ae. aegypti* populations by 60-80% with 85% area coverage (Barrera et al. 2014a,b). This reduction in vector population densities due to AGO deployment was correlated with a reduction in transmission of Chikungunya virus (Barrera et al. 2016). Similarly, AGOs were effective in controlling gravid *Ae. aegypti* with good public acceptance in Australia (Mackay et al. 2013, Ritchie et al. 2009, Rapley et al. 2009). In the current study, the SIRENIX trap appeared to outperform the AGO.

The present study adds the first field-based information on the SIRENIX trap as an effective mosquito control tool and increases information on the AGO trap as novel, cost-effective toolset, which can be used by mosquito control districts for integrated mosquito management. However, additional investigations during peak mosquito activity time would be useful for programs to understand the utility of these traps. Additionally, an evaluation using the recommended coverage rate of 3 traps per house, this study averaged 2.3 traps per house, would provide more valuable information for mosquito control programs and potential use in operational settings.

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OPERATION NOTE

LABORATORY EXPLORATION OF TOLFENPYRAD AND NATURECIDE IN TOXIC SUGAR BAITS AGAINST *AEDES AEGYPTI*

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ABSTRACT

Attractive toxic sugar baits (ATSB) are an effective mosquito control tool based on sugar feeding behaviors and oral ingestion. There is a demand from consumers for more effective active ingredients. Torac 15EC, containing tolfenpyrad, is a registered insecticide for control of agricultural pests, which may mean transferability to mosquito control. Another option, Naturecide Pest Management (NPM), is a botanical insecticide containing cedarwood and cinnamon oils for control of adult mosquitoes via contact. This study evaluated tolfenpyrad and a formulated essential oil blend added to toxic sugar baits (TSB) against adult *Aedes aegypti*, compared with a positive control of 1% boric acid toxic sugar bait, and untreated control. In this experiment setup, ingestions of tolfenpyrad at 1%, NPM at 1% (of finished product) TSB, and boric acid at 1% by female *Ae. aegypti* mosquitoes resulted in average mortality at 48 hrs were 71%, 73% and 95%, respectively. The results suggest that ingestible tolfenpyrad, Naturecide Pest Management, and boric acid TSBs may be potential tool for mosquito control strategies and programs, but the mode of action of essential oils to kill adult mosquitoes is still needed to be addressed.

Key Words: alternative control, mosquito, ATSB, mortality

INTRODUCTION

Aedes aegypti (L.) is a vector of yellow fever, dengue, chikungunya, and Zika. Vector control plays an important role in the reduction of the most mosquito-borne diseases due to lack of effective vaccine for prevention and drug for treatment. Attractive toxic sugar bait (ATSB), an alternative control strategy, has demonstrated a potential for resistance management (Pearson et al. 2020), and needs further research to identify optimal active ingredients (Davis, et al. 2021). Boric acid, spinosad, ivermectin, dinotefuran, and eugenol have all resulted in significant efficacy against a number of important adult mosquito species by oral administration with attractive sugar baits (Fiorenzano et al. 2017, Naranjo et al. 2013, Xue & Barnard 2003). Other additives, such as floral cues, fruit byproducts, attractants, or host kairomone additives also enhance the efficacy due to increase of attraction for adult mosquitoes to have more opportunity to ingest the toxins (Fiorenzano et al. 2017, Scott-Fiorenzano et al. 2017). However, there are still a number of other possibilities in formulation that may improve efficacy. For example, Torac 15EC contains tolfenpyrad, an electron transport chain inhibitor used in agriculture for soil and foliar insect management. This product may have transferability to mosquito control if it can kill adult mosquitoes in alternative public health formulations. Also, several essential oils present a large array of potential active ingredients, it may benefit the selection of specific ingredient profiles. However, previous research on an application rate at 70ml/L of Naturecide all purpose commercial concentrate in a semi-field ultra-low volume spraying evaluation resulted in 100% mortality of *Ae. aegypti* (Bibbs et al. 2019). In contrast, spraying Naturecide Pest Management (NPM) on vegetation in the field as a foliar residue also significantly reduced natural population of *Aedes albopictus* (Skuse) (Smoleroff et al. 2019). It is plausible that Naturecide formulated essential oils could serve as viable active ingredients in TSB as well. The current laboratory study serves as a preliminary examination of tolfenpyrad and Naturecide

Pest Management for use in ATSB against adult *Ae. aegypti*.

Adult, pesticide susceptible *Ae. aegypti* (1952 Orlando strain) were used in this experiment, were reared in the Anastasia Mosquito Control District insectary, St. Augustine, FL, USA and maintained at 26.6°± 1°C, 80±10% relative humidity, and a 14L:10D photoperiod. A 120 ml lidded transparent plastic feeding cup (P200N, Dart Container Corporation, USA) was filled with 100 mL test solution and a cotton wick was inserted to the cup through a hole in the cup lid. The feeding cup was nested into a lidded 0.5 L transparent plastic container with a small hole to introduce mosquitoes as described by Davis et al (2021). There was a total of three replicates, with a replicate composed of one container for boric acid sugar bait at 1% (Rose Mill, Hartford, CT 06110), one for tolfenpyrad at 1% of finished product (making ~0.15% tolfenpyrad; Nichino America, Inc., Wilmington, DE), one for Naturecide Pest Management (NPM) at 1% of finished product (making 0.25% cedarwood and 0.13% cinnamon oil; Pacific Shore Holdings, Inc., Canoga Park, CA), and one for 10% sucrose as negative control. To administer the bait treatments, a feeding cup was placed inside each of the large containers. Twenty adult female *Ae. aegypti*, aged 5-7 day old were transferred by a mouth aspirator into their respective large container and the hole was covered with cotton.

The boric acid TSB consisted of 1% boric acid and 10% sucrose in an aqueous solution. Boric acid was selected because it is safe and eco-friendly and has been used in previous TSB and ATSB evaluations (Naranjo et al. 2013, Xue & Barnard 2003, Xue et al. 2006). The control consisted of a 10% sucrose solution only. Both treatments and controls were administered by saturating cotton wicks in the solution. The study was a no-choice assay where mosquitoes were only provided either the TSB solutions (treatments) or 10% sucrose solution (controls). All testing containers were kept in an enclosed room maintained at approximately 24.4 °C with a 12L:12D photoperiod.

Mortality was read at 48 h following transfer to treatment cages. Dead mosquitoes in the large containers were counted each day. After 48h, the remaining live mosquitoes were counted after freezing for 30 min. The mortality data was correct by Abbott (1925) formula and analyzed using SPSS-version 20 (IBM® SPSS® Statistics). A two-way ANOVA was performed to determine any significant interactions between different toxic baits and treatment.

The boric acid toxic sugar baits resulted in higher mortality for *Ae. aegypti*. Tolfenpyrad resulted in comparably low mortality (71%) for adult mosquitoes at 48 h after ingestion. Average mortality at 48 h of adult mosquitoes, *Ae. aegypti* after ingestion of boric acid sugar baits were 95%. Average mortality at 48 h of adult mosquitoes after ingestion of Naturecide TSB were 73%. The control mortality remained below 10% for all cohorts. There were significant differences in mortality of adult mosquitoes at 48 h after ingestions of toxic sugar baits between treatment groups ($F2,8 = 4.932$, $P < 0.01$).

This study determined that the boric acid, tolfenpyrad, and Naturecide TSBs caused significant mortality of adult *Ae. aegypti*. Our results corroborate the earlier findings that boric acid (Naranjo et al. 2013, Xue & Barnard 2003) and essential oils (Revay et al. 2015, Traore et al. 2019) can kill adult *Ae. aegypti* mosquitoes after ingestion in a laboratory experiment and field (Revay et al. 2015, Xue et al. 2006).

In previous experiment, adult mosquitoes have been confirmed that all adult mosquitoes ingested the sugar and toxic sugar baits within 48 hours by different colors of dyes (Davis et al. 2021). Sugar baits and bait stations formulated with boric acid have been extensively evaluated against adult mosquitoes and consistently demonstrate high mortality in several species of adult mosquitoes (Barbosa et al. 2019, Fiorenzano et al. 2017, Naranjo et al. 2013, Xue & Barnard 2003). Liquid formulation of boric acid baits provided effective control for adult mosquitoes after being sprayed on plants (Naranjo et al. 2013, Xue et al. 2006). Our findings that the essential oil product NPM and the insecticide tolfenpyrad as TSB demonstrated a potential for inclusion in control of adult *Ae. aegypti* and worth investigating further.

This laboratory study demonstrated that NPM added to a TSB can provide significant control against adult *Ae. aegypti*. As with other ATSBs, development of essential oils and tolfenpyrad in toxic sugar baits could results in effective bait stations, or liquid solutions for direct application on vegetation. Further studies are needed in the field using attractants to explore the use of tolfenpyrad and essential oil TSB applications on wild *Ae. aegypti* populations.

Several essential oils in particular are very repellent, and many insects will choose to starve rather than feed on them (Lee 2018). The observed results could be solely due to starvation, or lack of feeding even if the active ingredients, cedarwood and cinnamon oils have not showed a strong repellency (Nerio et al. 2010). However, the mode of action of 0.25% cedarwood and 0.13% cinnamon oils as the NPM's active ingredient for the ATSB is still needed to be further addressed.

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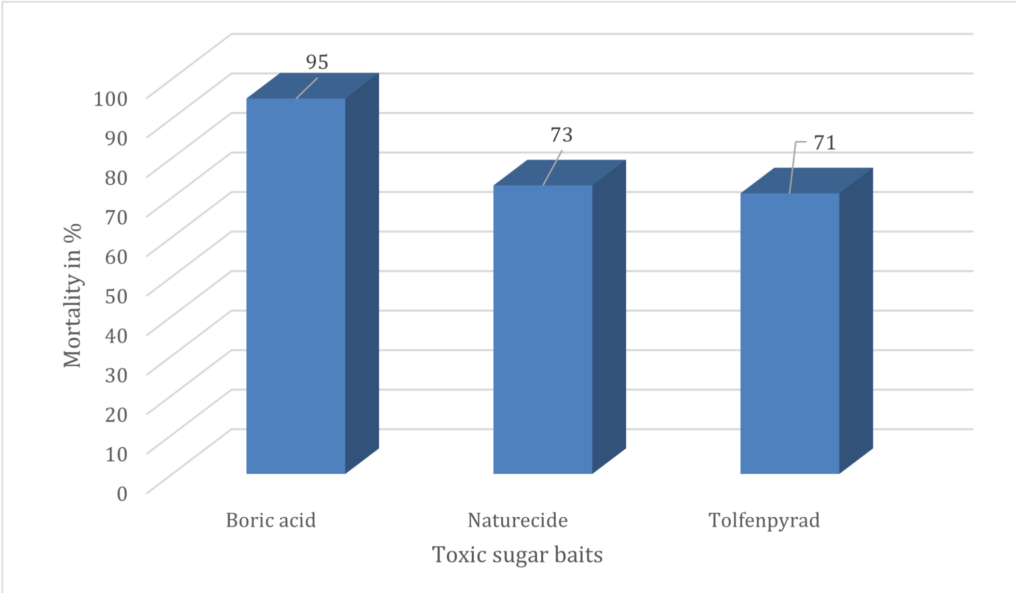

Figure 1. Mean mortality of 5-7 day old, adult, female, *Aedes aegypti* at 48 h after ingestion of different toxic sugar baits (boric acid 1%, naturecide 1% from product, and tolfenoyrad 1%).

providing assistance to complete this project and the MGK provided sample of tolfenpyrad. This is a research report only and specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District.

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OPERATIONAL NOTE

EFFECT OF VEGETATION PATTERNS ON EFFICACY OF A GROUND ULV SPRAY OF AQUARESLIN® AGAINST A NATURAL POPULATION OF *CULEX PIPIENS PALLENS*

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ABSTRACT

A field study was conducted to evaluate control efficacy of ground ultra-low-volume (ULV) applications (Aquareslin®) against a natural population of *Culex pipiens pallens* in three different vegetation areas (dense, open (grassland), and sparsely vegetated) in Changping county, Beijing City, China. Over 80% population decline rates (PDRs) were achieved from the different vegetation levels. Significantly higher PDR was achieved in the sparsely vegetated habitat compared to the dense and open habitats. Significant higher reduction (%) of parity in female mosquitoes was found in the open grassland and sparse vegetation level, compared with the dense vegetation level. Control efficacy of ground ULV spray against a natural population of *Cx. pipiens pallens* was impacted by the different vegetation levels.

Key Words: permethrin, ULV spray, habitats, vegetation pattern, parity

INTRODUCTION

Culex pipiens pallens Coquillett is a member of the *Cx. pipiens* complex (Zhao and Lu, 1994, Wu et al. 2014), which is distributed widely in the northern part of China and is considered a vector of *Wuchereria bancrofia* (Lu 1999). Source reduction and catch basin management are the primary methods for control of *Cx. pipiens pallens*. However, ground ultra-low-volume (ULV) applications of adulticides is still one of the important control measures in mosquito abatement programs around the world (Mount 1998).

Cx. pipiens pallens mosquitoes preferentially feed on birds and rest in vegetated areas (Lu 1999). Adult mosquitoes need vegetation for sugar feeding, water drinking, resting, and hiding (Xue 2008). The vegetation coverages directly relate to the population abundance of adult mosquitoes. The high number of mosquitoes usually resulted in the high number of service requests by residents (Davidson et al. 2016, Davis et al. 2022). A few field experiments have shown that the presence of vegetation influenced the adulticiding efficacy (Linley & Jordan 1992, Floor et al. 1991, Lothrop et al. 2002, Barber et al. 2007). Focks et al. (1987) documented that gravid female mosquitoes remain sequestered during treatment periods in places that are well protected from aerosols. Lothrop (2002) and Barber et al. (2007), confirmed that vegetation density impacted the control efficacy of ground sprays of permethrin and malathion against caged salt marsh mosquitoes.

The objective of the current study was to determine the effectiveness of the Ultra-Low-Volume (ULV) spray of Aquareslin®, a permethrin product against a natural population of *Cx. pipiens pallens* and the parity states of the female mosquitoes collected from the different vegetation density areas.

The site (5.59 hectares) located in the east part of the Changping county (N40.2161, E116.2347), Beijing, China was chosen for this study. The vegetations in the area are primarily *Bambusauentricosa multiplex* L., *Juniperus chinensis* L., *Sophora japonica* L., *Pinus tabulaeformi*s Carr., and *Salix babylonica* L.

The area was divided into 3 sections based on vegetation coverage: dense (>80% coverage) sparse (approximately 30%), and open grassland. The 3 sections were next to each other with about 100 meter distance (Fig.1). The experiment was conducted at 19:30 hrs, just after sunset, in late July. Air temperatures ranged from 30.5°C to 31.5°C with a relative humidity of 50%. Wind velocity was measured with a hand-held wind meter and recorded as consistent winds of 0.7m/s during the application.

Aquareslin® with the active ingredients (A.I.) S-esbiothrin, permethrin, and PBO at 1.5:108:110 g/L was provided by Bayer Company, Beijing Branch, China and diluted at 1:9 (1 part of Aquareslin and 9 parts of tape water) before the ULV spray. The ground ULV spray rate of 15.39g AI/ha was used. A truck-mounted DYNA L30 cold aerosol generator (Curtis DYNA-Fog, Jackson, GA 30233) was used to spray the mixture materials. The spray route was designed to make sure that the Aquareslin ULV spray covered all of the habitats. The trucks traveled at a speed of 8 km/hour. Flow rate calibrations were completed prior to the treatments, and the rate of output was 590 mL/minute. The three different vegetated areas were treated by spray of the same adulticide at the same time. A fifty-meter buffer zone around each of the three vegetation areas was designated as a quarantine zone. The most upwind area in relation to the three treated areas was designated to be the untreated control area.

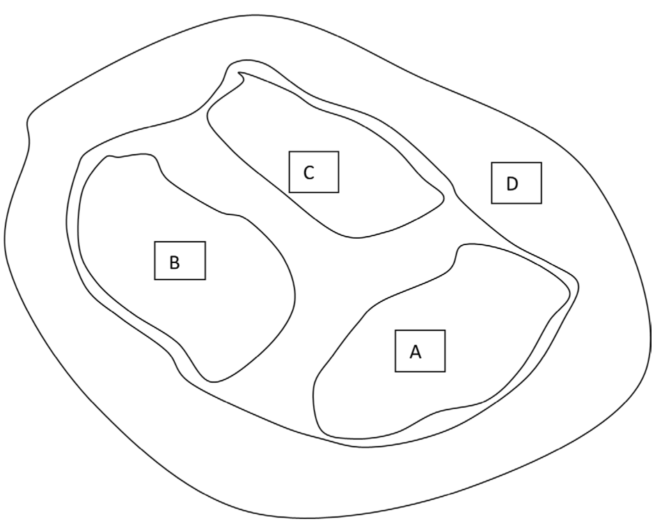

Figure 1. Diagram of the study site showing the distribution of different sections, based on vegetation pattern as well as buffer zones. A: dense vegetation area. B: sparse vegetation area; C: open grassland area. D: quarantine zone as control.

Adult mosquitoes were collected with a portable battery-operated aspirator in a mosquito net (top: 80×80 cm^2, bottom:150×150cm^2, H:120cm) hung a half meter above the ground. A volunteer (the volunteer signed the consent form and accepted the guidelines established by the institute handbook on risk associated with mosquito exposure) was in the net as the human bait to attract mosquitoes. Three collection sites were selected at central locations within each vegetated area. The pre-collection was conducted half an hour before the ULV application and post-treatment collections were conducted half an hour post application. Collection at each area was conducted for 20 minutes. All collected mosquitoes were brought to the laboratory for species identification and dissected for the parity evaluation. *Cx. pipiens pallens* was the main species collected from the all areas.

The control efficacy of ground ULV spray of Aquareslin® against a natural population of *Cx. pipiens pallens* was justified by the relative population index (RPI) and population decline rates (PDR) after spray, compared with the RPI and PDR before the spray. The RPI and PDR were calculated using the following formula:

$$RPI = \frac{Cb \cdot Tp}{Cp \cdot Tb}$$

Cb: mean density of mosquitoes in control area before treatment.

Tp: mean density of mosquitoes in test area after treatment.

Cp: mean density of mosquitoes in control area after treatment.

Tb: mean density of mosquitoes in test area before treatment.

PDR=(1-RPI)x100%

PDR: population decline rate (%)

The female mosquitoes collected were dissected under a microscope for parity status (parous and nulliparous) in the laboratory based on the description by Detinova (1962).

The data was analyzed by multiple way ANOVA and the means were separated by Tukey's HSD test.

After the ULV spray, the RPIs of *Cx. pipiens pallens* in dense vegetation area, open grassland, and sparse vegetation area were 0.16, 0.15, and 0.04, respectively (F =23.271, df=2, and P<0.05(Table 1). Therefore, the PDRs of *Cx. pipiens pallens* were 84% in dense vegetation area, 85% in open grassland, and 96% in the spare vegetation area, respectively. The percent PDR of mosquitoes caused by the ULV spray in the sparse vegetation area was significantly higher than the rates obtained from the open grassland and dense vegetation area (P<0.05). The results indicated that the droplet clouds of Aquareslin® could penetrate through the sparse vegetation pattern and a majority of the mosquitoes were killed in this area. Rathburn et al. (1989) proved that more droplets of insecticides were sampled in the open area than in the vegetated area. Other studies found that insecticide applied in moderately vegetated residential areas were less effective than in the open area with caged laboratory-reared mosquitoes (Floore et al. 1991, Baber et al. 2007). However, it was surprising that the Aquareslin® ground ULV spray in the open grassland area did not result in a significantly higher PDR, compared to the rate in the sparse vegetated area in our experiments. Migrating mosquitoes from the adjacent vegetated area may have caused the low value of the PDR of mosquitoes in open grassland. Also, the wind in the open grassland area may play a major role due to non-vegetation barrier impact during the application of Aquareslin®. This has been documented by Inman et al. (1997). The higher PDR of mosquitoes in the sparse vegetation area may have been caused by the Aquareslin® ULV spray deposited on the vegetation. The insecticides on vegetation may result in more mortality of adult mosquitoes through the contact.

The efficacy of an Aquareslin® ground ULV spray on parous rates of a natural population of *Cx. pipiens pallens* was justified by the percent parous and nulliparous female mosquitoes after the treatments, compared with the rates before conducting the ULV spray. The parous rates in the female mosquitoes before and after the ULV spray in dense vegetation area, open grassland, sparse vegetation area, and control area are presented in Table 2. After the ULV spray, the parous female mosquitoes in the open grassland area were significantly reduced (F=36.75, df = 1, P <0.05). Our field experiment showed a 100 % parous reduction in the natural population of *Cx.*

Table 1. The number of mosquitoes captured before and after an application of Aquareslin® ground ULV spray by each vegetation pattern in Changping, Beijing, China.

	Before treatment	After treatment	RPI
Dense vegetated	199	105	0.1607
Open grassland	104	9	0.1538
Sparse vegetated	76	39	0.0264
Control	12	39	

Table 2. The effect of an Aquareslin® ULV spray on parous rates (%) of female Culex pipiens pallens in three different vegetation patterns in Changping, Beijing, China.

	Before treatment			After treatment		
	Parous	Nulliparous	Parous Rate	Parous	Nulliparous	Parous Rate
Dense vegetated	12	31	27.9	9	21	30
Open grassland	7	15	31.8	0	10	0
Sparse vegetated	12	20	37.5	1	2	33.3
Control	3	8	27.3	7	21	25

T =1.176, df= 3, P = 0.325

pipiens pallens after the Aquareslin® ULV spray and these results support the observations by Zeng et al. (1991). Higher mortality in parous female *Cx. pipiens pallens* were recorded based on the Deltamethrin and Cypermethrin (Fendona) treatment, compared to the mortality in nulliparous mosquitoes (Zeng et al. 1991). Theoretically, parous mosquito and nulliparous mosquitoes had equal chances to be killed or survive. Some mosquitoes survived because they rested in secluded places, such as beneath tree bark and underneath leaves. Since there were fewer resting sites in the open grassland area, most mosquitoes collected in this area were migrating from neighbouring treated areas. Low parous mosquito collections after treatment in the open grassland area may indicate that nulliparous mosquitoes were more active in blood feeding than the parous ones.

Different vegetation patterns in treated areas affected the control efficacy of a ground Aquareslin® ULV spray against a natural population of *Cx. pipiens pallens.* Also, the ULV spray provided effective control for parous female mosquitoes in the open area.

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SCIENTIFIC NOTE

USING BG LURE VERSUS NON-ATTRACTANT IN STICKY TRAPS TO EVALUATE EFFECTIVENESS OF COLLECTING EYE GNATS (DIPTERA: CHLOROPIDAE: *LIOHIPPELATES* SPP.)

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Subject Editor: Derrick Mathias

ABSTRACT

Increased number of eye gnats often cause a nuisance problem in St. Johns County, Florida, during spring and summer. Anastasia Mosquito Control District, St. Augustine often receives service requests and complaints about the eye gnat problem. Evaluation of traps is critically important for surveillance and control of nuisance eye gnats. This study was conducted to determine if sticky traps (RESCUE!® TrapStik for Flies) baited with BG Lure were more attractive to eye gnats than sticky traps without bait in Elkton, northeastern Florida. The results showed that sticky traps baited with BG Lure were more attractive to eye gnats than unbaited traps, which could be considered as a tool for surveillance and control of eye gnats.

Key Words: sticky trap, BG Lure, eye gnats, Liohippelates, Diptera, Hymenoptera, Coleoptera, Hemiptera, Homoptera, Thysanoptera, spiders

INTRODUCTION

The two most common species of eye gnats in the southeastern United States are *Liohippelates pusio* (Loew) and *L. bishoppi* (Sabrosky) (Klepzig et al. 2022). These non-biting flies are attracted to fluids secreted from the eyes, nose, ears, and open wounds of both humans and animals (Mulla 1965). As flies feed on these fluids they can transmit pathogens such as bacteria and viruses between and among humans and livestock. Where eye gnats are abundant, they become a nuisance to humans in rural towns and tourist and agricultural areas. Eye gnats have been implicated in the mechanical transmission of several disease-causing pathogens including the bacteria that cause human acute conjunctivitis (pink eye) (Machtinger and Kaufman 2011). Increased number of eye gnats often cause a nuisance problem in St. Johns County, Florida, during spring and summer, leading to Anastasia Mosquito Control District, St. Augustine receiving service requests and complaints. For eye gnat control, applications of insecticides have shown various degrees of success. Unfortunately, many of the effective chemicals are not labeled for eye gnat control in the U.S. The use of removal trapping (Day and Sjogren 1994) and attractants (Hwang et al. 1976) might be a promising alternative for the management of eye gnat populations.

During the Florida summer, the complete life cycle from egg to adult takes approximately three weeks (Klepzig et al. 2022). Primary breeding sites are freshly disturbed soil with cut grass or hay and moisture (Bigham 1941). This study was conducted to determine if a commercial sticky trap baited with an attractant was more productive for collecting eye gnats than traps without an attractant.

Sticky traps (RESCUE!® TrapsStik for Flies, Manufactured by Sterling International, Inc., Spokane, WA; Figure 1A) were used to assess the attractiveness of eye gnats (Chloropidae: *Liohippelates* spp.). This sticky trap uses a combination of appealing colors, patterns, and contrast to attract nuisance flies (Zhang et al. 2015). For this study, BG Lure (consisting of ammonia, lactic acid and fatty acids, BioGents, Regensburg, Germany; Figure 1B) was added as an attractant to the sticky traps to see if the lure improved trap performance at collecting eye gnats compared to an unbaited sticky trap. Also, all insects and spiders caught by the traps were identified and evaluated to determine the impact of baited and unbaited traps on non-target organisms.

The entire trapping study was repeated three times during the last week in August and the first two weeks in September 2019, each consisting of 3 pairs of sticky traps with BG lures vs. sticky traps without BG lures. All six traps in each test were deployed in the field for 24 hours and then collected. A total of 18 traps, nine traps baited with BG Lures (n=9) vs. nine unbaited traps (n=9), were deployed during this study. The study area was in Elkton (Latitude: 29° 46’44.96” N; Longitude: -81° 26’ 12.24” W), St. Johns County, Florida.

All insects and spiders (Araneae) collected on the sticky traps were identified to order. Where possible, some taxa were identified to the lower taxonomic ranks of family or genus (Table 1). Only the most abundant orders were compared between the baited and un-baited traps (Table 2) using a *t*-test at $\alpha = 0.05$.

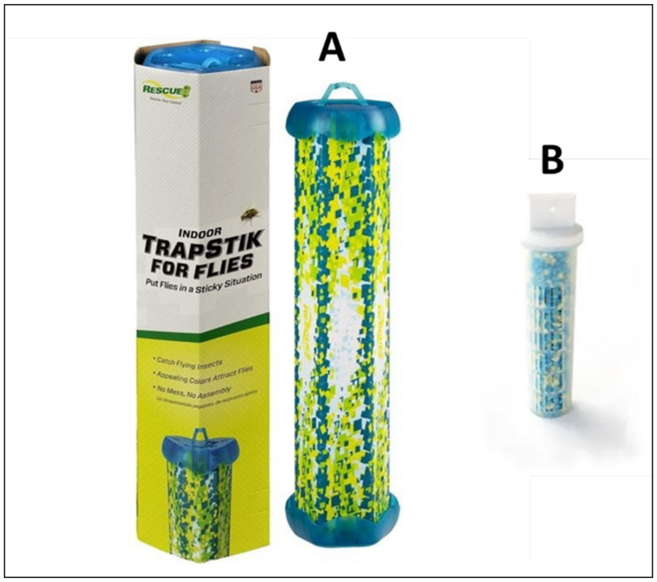

Figure 1. RESCUE!® TrapStik for Flies (A) and BG Lure (B) used in the field trapping experiment

A total of 2,408 specimens were collected during this study (Table 1). The BG Lure baited traps collected 1,318 specimens and the unbaited traps collected 1,067 specimens (Table 2). Figure 2 shows the difference in the numbers of *Liohippelates* spp. and the specimens from most common orders collected between the BG Lure baited and non-baited sticky traps during this study. The BG Lure traps were significantly more efficient at collecting *Liohippelates* spp. than the non-attractant traps ($t = 2.615$, $df = 8$, $p < 0.05$). Other dipterans were weakly but not significantly attracted to the BG Lure ($t = 1.438$, $df = 8$, $p > 0.05$). There were no significant differences in trap catch for all other orders between the BG Lure-baited traps and the unbaited traps, even though both traps caught substantial numbers of specimens (100-300 individuals) of these orders.

The BG Lure, consisting of ammonia, lactic acid, and fatty acids, was designed and marketed for BG traps to attract container-inhabiting *Aedes* mosquitoes (Geier et al. 2004). In the current study, the combination of this lure with the commercial sticky trap (RESCUE!® TrapStik for Flies) attracted significantly more *Liohippelates* spp. than did the unbaited sticky traps. BG lures did not show significant attraction to other filth flies nor to other orders of insects or spiders when compared to the catch in unbaited traps. However, both sticky traps (baited and unbaited) caught a great number of other dipterans, coleopterans, hymenopterans, thysanopterans, and spiders among others. This is probably due to the strong visual attraction of these insects to the blue/green color

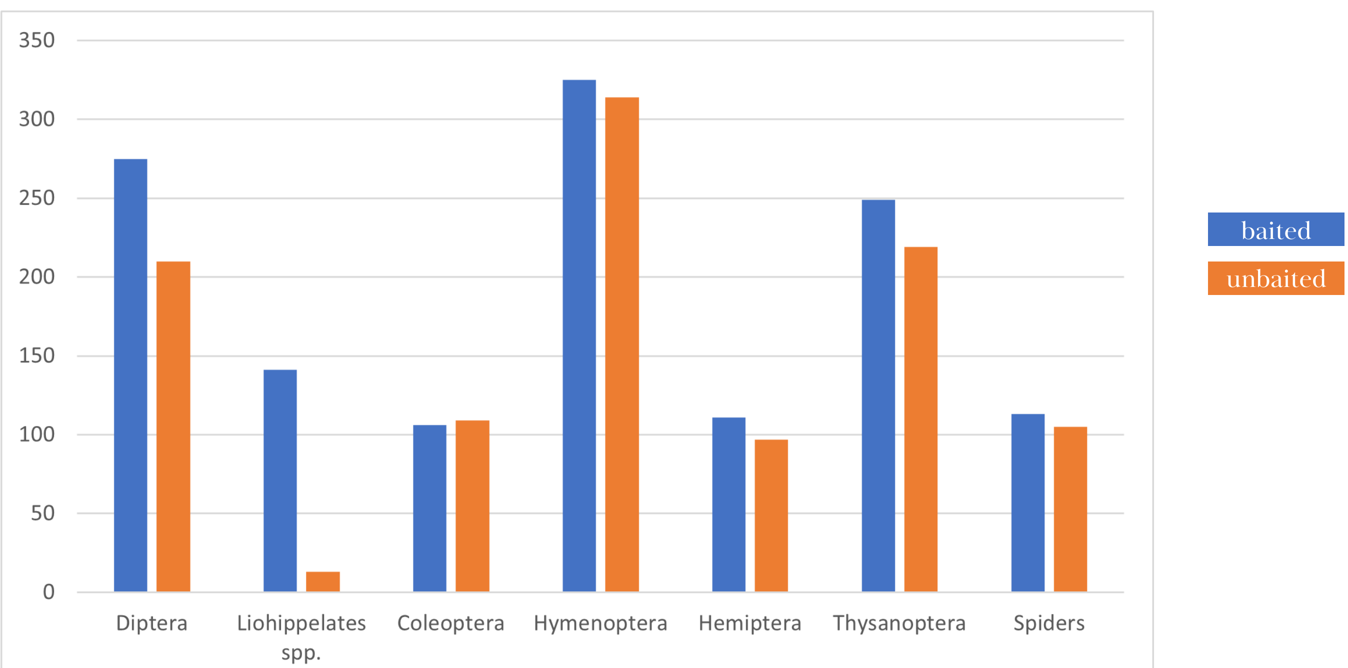

Figure 2. Total number of specimens captured by sticky traps baited with BG lure vs. without lure.

combinations, clustering pixel patterns and strong color contrasts of the RESCUE!® TrapStik for Flies (Zhang et al, 2015).

As with most sticky traps a large number of non-target insects were collected. Non-target species accounted for 93.8% of the total trap catch. Future studies could consider using different (and more selective) types of traps such as Biogents Sentinel Traps baited with dry ice and/or BG Lure. This approach would take advantage of eye gnat behavior if they are attracted by CO2 first (Defoliart and Morris 1967) and then to host odors as they search for tears and other moist areas of the host. Nevertheless, discovery and development of more powerful attractants and traps are needed for optimal monitoring/surveillance and control of these important nuisance eye gnats.

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Table 1. Identified taxa and number of specimens collected. For each order, the first number in the last column refers to the number of specimens only identifiable at that taxonomic rank, while the number in parentheses refers to the total number of specimens identified at all levels within the order.

Taxonomic Rank				No. of Specimens
Class	**Order**	**Family**	**Genus**	
Insecta				
	Blattodea			0 (2)
		Blattidae		2
	Coleoptera			172 (215)
		Carabidae		22
		Curculionidae		5
		Elateridae		2
		Scarabaeidae		2
		Scolytidae		1
		Staphylinidae		11
	Diptera			474 (637)
		Bombyliidae		3
		Chloropidae	*Liohippelates*	149
		Culicidae		1
		Platystomatidae		3
		Syrphidae		6
		Tabanidae		1
	Hemiptera			42 (208)
		Aphididae		1
		Cercopidae		1
		Cicadellidae		63
		Cydnidae		90
		Reduviidae		8
		Scutelleridae		3
	Hymenoptera			605 (639)
		Apidae	*Apis*	2
		Formicidae		28
		Sphecidae		1
		Vespidae		3
	Lepidoptera			6 (14)
		Hesperiidae	*Urbanus*	1
		Papilionidae	*Papilio*	5
		Pyralidae		1
		Tortricidae		1
	Megaloptera			1 (1)
	Orthoptera			0 (2)
		Acrididae		1
		Tettigoniidae		1
	Psocodea			3 (3)
	Thysanoptera			468 (468)
Arachnida				
	Araneae (spiders)			217 (218)
		Salticidae		1
Collembola				
	Collembola (springtails)			1 (1)

Table 2. Total number of specimens for traps with and without BG Lure for the most common arthropod orders and the eye gnat genus *Liohippelates.* Numbers for the Diptera row do not include eye gnats

Taxonomic Rank			
Order	**Genus**	**BG Lure**	**Without BG Lure**
_Coleoptera		106	109
Diptera		278	210
	Liohippelates	136	13
Hemiptera		111	97
Hymenoptera		325	314
Thysanoptera		249	219
Aranae (spiders)		113	105
Total Specimens		1318	1067

OPERATION NOTE

ADULTICIDAL AND LARVICIDAL IMPACTS OF THE MIXTURE OF *BACILLUS THURINGIENSIS ISRAELENSIS* AND BORIC ACID TOXIC SUGAR BAIT (TSB) AGAINST *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Toxic sugar baits (TSBs) can be used to deliver insecticide material via ingestion instead of via contact through spraying of insecticides by targeting the resting and sugar-feeding behaviors of adult mosquitoes. This semi-field study aimed to evaluate the adulticidal and larvicidal dual action of a foliage spray of a TSB with a mixture of *Bacillus thuringiensis israelensis* (Bti), and boric acid against *Aedes aegypti* Puerto Rico (PR) strain (resistant) and Orlando (OR) strain (susceptible), and laboratory colony of *Culex quinquefasciatus* (Gainesville 1995 strain). The larval and adult evaluation of TSB consisted of 11% VectoBac (Valent Biosciences, Libertyville, IL; Bti), 5% boric acid, and 10% sucrose solution. The TSB intervention for adults consisted of the same as the larval intervention but had an addition of a food grade, 5g Blue No. 1 dye (Sigma Aldrich; St. Louis MO) to observe adult feeding. The controls received a 10% sucrose solution. For the larval mortality evaluation, the TSB was applied to the bromeliad with the runoff dripping into pans containing mosquito larvae. At 24 hours post-application, 100% larval mortality was observed. At all-time mortality recordings, 50 larvae were introduced into the larval pans and the bromeliads were sprayed with water to mimic rainfall allowing the remaining TSB to be washed off into the larval pans. After the 4th day of larval introductions, larval mortality was 83.5% ± 14.3 for *Ae. aegypti*_PR, 92.5% ± 6.1 for *Ae. aegypti* OR, and 97 % ±1.7 for *Cx. quinquefasciatus.* Total mean mortality at 72 hours post exposure for the adult TSB evaluation was 52.7% ± 24.2 for *Ae. aegypti*_PR, 34.3% ± 26.5 for *Ae. aegypti*_OR, and 73.7% ± 13.9 for *Cx. quinquefasciatus.* Our study suggests this TSB including Bti is effective against larvae when applied as an adulticide barrier application and could be a dual-action approach to mosquito control.

Key Words: *Aedes aegypti*, *Culex quinquefasciatus*, toxic sugar bait, dengue vector, *Bacillus thuringiensis israelensis*, boric acid

Aedes aegypti L. and *Culex quinquefasciatus* Say are important vectors of diseases impacting global public health (Daep et al. 2014, Alto et al. 2016). Both the geographic distribution and insecticide-resistant populations of these species are rapidly spreading and due to their public health burden as vectors of dengue and West Nile virus (respectively among others), novel control methods are more imperative than ever (Kraemer et al. 2015, Liu et al. 2020). Insecticides are a common control method for reducing vector-borne disease transmission but the development of resistance to these insecticides is an ever-present challenge for control programs (Namias et al. 2021). Additionally, there are limited effective mosquito control insecticides with a need to identify different insecticide formulations or delivery methods for mosquito management. A new alternative for mosquito control is the use of toxic sugar baits (TSB) (Xue et al. 2006, Pearson et al. 2020, Davis et al. 2021).

The application of TSBs to vegetation exploits the resting and sugar-feeding behaviors of adult mosquitoes instead of the flying/host seeking mosquitoes targeted by truck/ aerial adulticide applications. TSBs deliver the toxic material via ingestion instead of via contact through spraying of larvicides and adulticides. TSBs can also be formulated to be attractive (ATSB) to the adult mosquito, likely increasing ingestion of the toxic material resulting in increased adult mosquito mortality (Qualls et al. 2014). Most TSBs and ATSBs evaluations have been performed as barrier sprays (Qualls et al. 2014).

Barrier applications are an effective strategy for mosquito control due to their residual nature, but the insecticide residual is impacted by rain events (Allan et al. 2009), reducing the effectiveness over time. In an effort to combat the loss of adulticidal residual of barrier applications through exposure to rain events, one thought has been to benefit from rain events by combining the

TSB/ATSB with a larvicide (Fulcher et al. 2014, Scott, et al. 2016). *Bacillus thuringiensis israelensis* (Bti) is a common larvicide and is most effective when used as part of an integrated mosquito management program (IPM) (McAllister et al. 2020). A recent study demonstrated that 8% Bti combined in a TSB is successful in controlling adult, female *Ae. aegypti, Aedes albopictus* (Skuse), and *Cx. quinquefasciatus* resulting in an average mortality of 97%, 98%, and 100%, respectively at 48 h (Davis et al. 2021). To follow up on the work by Davis et al. (2021), a semi-field study was conducted to investigate the adulticidal and larvicidal dual action of a foliage spray of TSB combining Bti, and boric acid against *Ae. aegypti* and *Cx. quinquefasciatus.*

Mosquitoes. Two strains of *Ae. aegypti*; the resistant Puerto Rican strain (*Ae. aegypti*_PR) and the susceptible Orlando 1952 strain (*Ae. aegypti*_OR), and *Cx. quinquefasciatus* Gainesville strain were used in this experiment. The eggs of all strains were acquired from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. All mosquitoes were maintained as colonies at Anastasia Mosquito Control District (AMCD), St. Augustine, Florida's insectary, at 26.6 ± 1°C, 70.0 ± 10% relative humidity, and a 14:10 light: dark (LD) photoperiod.

Larvicidal experiment. Fifty, 2nd-3rd instar larvae of each strain were pipetted into plant saucers containing 500mL of reverse osmosis (RO) water that was placed underneath a bromeliad plant (Bromeliaceae; 3.7 liter pot from Home Depot Store, St. Augustine, FL). The bromeliads and plant saucers were housed inside bug dorms (BioQuip, Rancho Dominguez, CA, 30x30x30 cm.) within the AMCD greenhouse (26.6 ± 1°C, 70.0 ± 10% relative humidity). Three control and three TSB treated bromeliads were placed in separate bug dorms for this evaluation. The TSB intervention consisted of 11% VectoBac (Valent Biosciences, Libertyville, IL; Bti), 5% Boric Acid (Sigma Aldrich, St. Louis, MO), and 10% sucrose solution. Boric acid has been commonly used as a toxicant in the laboratory in TSBs and ATSBs efficacy evaluations against adult mosquitoes (Xue et al. 2006, Pearson et al. 2020). The control plants received a 10% sucrose solution applied to the vegetation. Both the control and intervention solutions were administered by a 1-liter ZEP professional sprayer bottle (Zep Inc. Atlanta, GA) saturating the bromeliad plants until the solution was dripping from the plants (~ 25 ml) into the plant saucer that contained the larvae. Mortality was documented at 24-, 48-, 72-, and 96 h. Dead larvae were removed from the control and intervention saucers at each time period, and 50 more larvae (second to the third instar) were added each day to the saucer up to four days post-treatment. At each time period after 24 h, both control and intervention bromeliads were sprayed seven times with RO water to provide solution runoff into the saucers. The spraying of RO water was to mimic a rain event for observing the longevity of the TSB treated foliage, and to measure the residual TSB effects on larval mortality. The experiment was repeated three times.

Adulticidal experiment. One hundred, five-to seven-day-old male and female adults of each strain were aspirated into bug dorms held in the AMCD greenhouse (26.6 ± 1°C, 70.0 ± 10% relative humidity). Three control and three TSB treated cups containing cotton balls were placed in bug dorms as described above for this evaluation. The intervention cotton balls received a TSB consisting of 11% VectoBac (Bti), 5% Boric Acid, 5g of Blue No. 1 dye (Food grade dye; Sigma Aldrich; St. Louis MO), and 10% sucrose. The 5g of food grade dye was used so that only the dyed, dead mosquitoes were counted for the confirmation of the ingestion on the TSB. The food grade dye was used just in the intervention treatment. The control cotton balls received a 10% sucrose solution. Both the control and intervention solutions were administered by saturating cotton balls in the solution and placing them in a 60 ml plastic cup in each cage. The bromeliad was still included in the cages because it is a common plant and breeding site in residential areas and homes in Florida. Mortality was documented at 24-, 48- and 72 h. Dead mosquitoes were aspirated from the bug dorms at each time period. The experiment was repeated three times.

Data analysis. The data was analyzed using SPSS-version 20 (IBM SPSS Statistics). Data sets were arcsine transformed and independent t-tests were performed to determine any significant difference between the control and treatment groups of each species/strain at each time period. One-way ANOVA with Tukey's pairwise multiple comparison tests were also performed to compare the TSB mortality of each species at different time periods and between species/strains.

The larvicidal effect of the TSB resulted in 100% mortality at 24 h for all larval species/strains tested (Figure 1). After four days of larval introductions, the total mean mortality was 83.5% ± 14.3 for *Ae. aegypti*_PR, 92.5% ± 6.1 for *Ae. aegypti*_OR, and 97 % ± 1.7 for *Cx. quinquefasciatus.* The control mortality remained below 5% for all three species/strains at each time period and were significantly lower than corresponding TSB mortality ($P<0.05$ for all). There were no statistically significant differences between the different time periods for the species/strain. The ANOVA performed on data

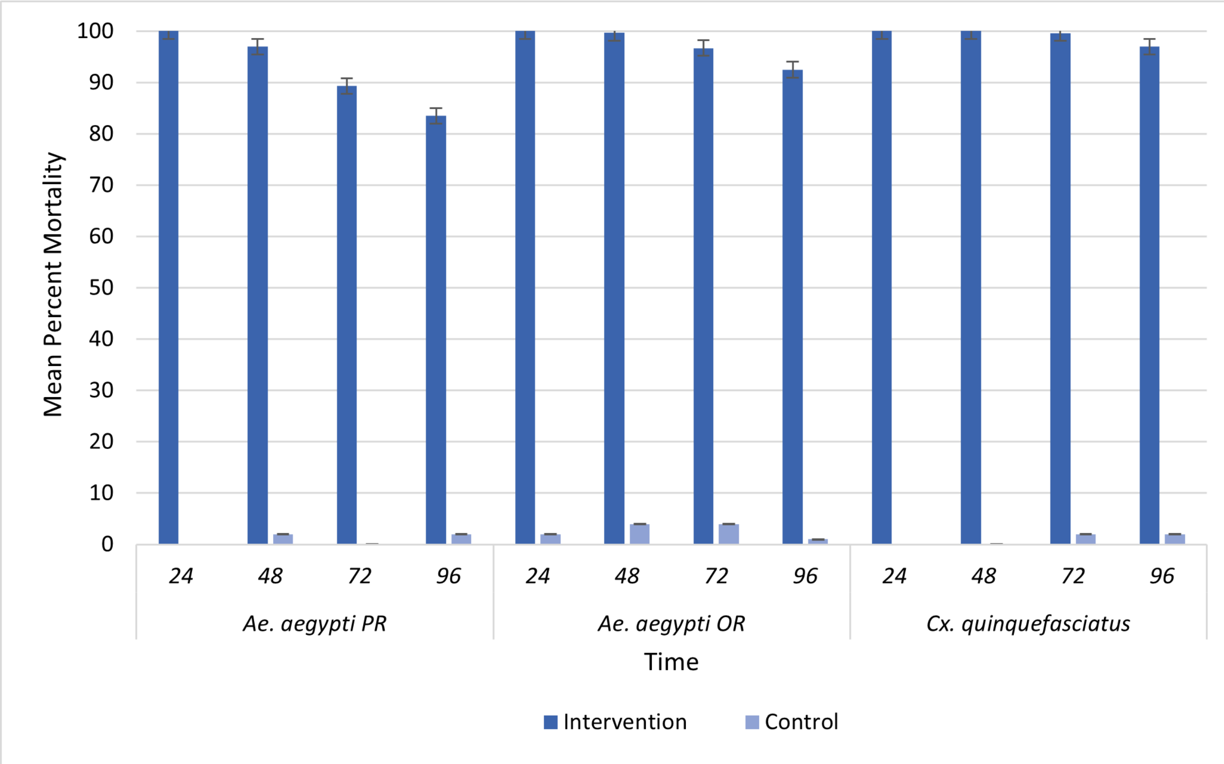

Figure 1. Cumulative mean larval mortality of the three species/strains at 24, 48, 72, and 96-h time periods following wetting events of 25 ml of RO water to simulate run-off of the barrier TSB application. (error bars=standard error)

sets pooled for time periods did not show any significant differences in mortality between species/strains with F_2 = 1.09, and P = .0342.

The TSB resulted in adult mortality at each time period for all three species/strains tested (Figure 2). At the 72-h time check, the total mean mortality for all days was 52.7% ± 24.2 for *Ae. aegypti*_PR, 34.3% ± 26.5 for *Ae. aegypti*_OR, and 73.7% ± 13.9 for *Cx. quinquefasciatus*. The control mortality remained below 5% for all three species/strains. The TSB had significantly higher mortality at all time periods for the three species/strains compared to the control (Table 1) PR F_2 = 1.68 and P = 0.0264, OR F_2 = 1.66 and P = 0.268, Cx F_2 = 2.1 and P = 0.0204, except *Ae. aegypti*_PR at 72 h, *Ae aegypti*_OR at 24 h and 72 h, compared to controls. There were no statistically significant differences in mortality between different time periods of any species/strain. Analysis of data pooled for all time period revealed a significant difference in mortality between species/strains (P=0.015) and post-hoc tests confirmed the difference was only between *Cx. quinquefasciatus* and *Ae. aegypti*_OR (P=0.11).

This study demonstrated 100% mortality for all three species/strains of larvae at the initial treatment of the TSB. Although larval mortality decreased each post-treatment check, the mortalities of all three species were still high at the four days post-treatment count. The mortality count demonstrates the high residual effects of the TSB foliage spray and its impacts on larval development but further evaluations are needed to demonstrate if this translates over to effective field control. There could be reduced larvicidal impact of the TSB following rain events and would most likely be due to the dilution of the active ingredients in the TSB after each rain event. We demonstrate that continued larval mortality is possible with TSB foliage applications following natural wetting events.

The mortality of the adult mosquitoes was significantly lower in this study compared to previous literature results (Xue et al. 2006, Davis et al. 2021). This study concluded lower cumulative mean mortality of adult mosquitoes of the three species/strains at 72 h when compared to other studies. This study administered the TSB to the male and female adult mosquitoes by saturating cotton balls in the solution and placing them in a 60 ml plastic cup in each cage. Similar studies evaluated this TSB (Xue et al. 2006, Davis et al. 2021,) administered the TSB by spraying the

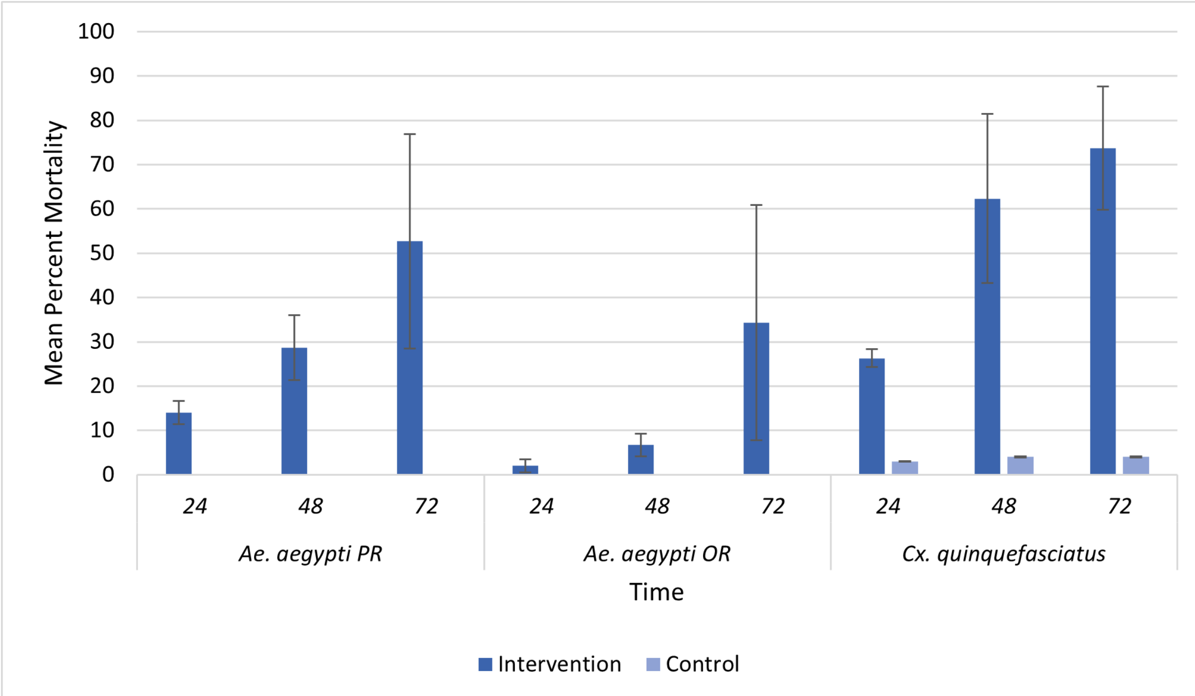

Figure 2. Cumulative mean adult mortality of the three species/strains at 24, 48, and 72-h time periods following exposure to a TSB solution (11% VectoBac (Bti), 5% Boric Acid, 5g of Blue No. 1 dye (Food grade dye; Sigma Aldrich; St. Louis MO). The control received only a 10% sucrose solution. (error bars=standard error)

foliage in the mosquito cages with the solution, possibly being the reason for the differences in mortality observed in the current study. The difference in how the TSB was administered may have altered the ingestion of the toxic solution based off the mosquito's typical resting habits being on foliage and the bromeliad plant may impact the mortality of adult mosquitoes (Xue et al. 2018). The adult TSB evaluation presents a few limitations as there was not a positive control for both the adult and larval trials. In future studies, trials need to be conducted to evaluate the impacts on adult mortality using boric acid and a sugar solution excluding Bti, and Bti and a sugar solution excluding boric acid.

Importantly, the findings demonstrate that adding a larvicide to a TSB can result in larval mortality if larvae are in the surrounding habitat. The results are similar with previous reports by Fulcher et al. (2014) and Scott et al. (2016) about TSB and the insect growth regulator, pyriproxyfen against *Ae. albopictus.* Future studies are planned to demonstrate the concentration of the larvicide to be added to the TSB for effective larval control in settings where the TSB may be flushed into storm drains or ditches.

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Table 1. Statistical significance of adult TSB mortality compared to control mortality at all three time periods for the three species/strains.

	24 hr	48 hr	72 hr
PR	$t_{(4)} = 10.07$	$t_{(4)} = 6.60$	$t_{(4)} = 2.64$
	P = 0.001	P = 0.022	P = 0.118
OR	$t_{(4)} = 1.66$	$t_{(4)} = 4.34$	$t_{(4)} = 1.85$
	P = 0.171	P = 0.012	P = 0.206
Cx	$t_{(4)} = 7.169$	$t_{(4)} = 3.10$	$t_{(4)} = 4.23$
	P = 0.002	P = 0.036	P = 0.013

t=test value, P=probability of significance

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SCIENTIFIC NOTE

EFFECTIVENESS OF A COMMERCIAL COMPETITIVE ELISA FOR THE DETECTION OF WEST NILE VIRUS ANTIBODIES IN SENTINEL CHICKENS

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ABSTRACT

The use of sentinel chickens is a powerful tool to aid in arboviral surveillance efforts. Results from this type of surveillance is used to help guide abatement efforts for vector species. Many mosquito control programs use outsourced laboratories for testing sentinel chicken samples. This study evaluated the use of a commercially available competitive enzyme-linked immunosorbent assay (cELISA) for in-house testing for West Nile virus antibodies from chicken serum. The commercial cELISA detected more positive sentinel chickens compared to the outsourced laboratory results. Results obtained from the in-house testing of chicken serum were reported sooner compared to the outsourced laboratory results, enhancing mosquito control programs timeliness for response to mosquito-borne disease.

Key Words: Antibody; chicken serum; ELISA; sentinel; West Nile virus

Vector-borne diseases remain a major human, veterinary, and wildlife health threat globally, and monitoring transmission is a critical process in the management of such diseases (Langevin et al. 2001). Mosquito control programs are tasked with the control and prevention of arboviral activity within their communities. In the United States, West Nile virus (WNV) is the most prevalent mosquito-borne disease and leads to human morbidity and mortality annually (Danforth et al 2021). As treatment and prevention options are limited for WNV, control of the pathogen vector is most effective in the reducing disease. Screening sentinel chickens for viral activity provides the relevant authorities the knowledge needed to guide their vector abatement decisions (Olson et al. 1991, Johnson et al 2003, Van Den Hurk et al. 2012, Day et al. 2015). Studies have shown that sentinel chicken surveillance can be one of the most sensitive indicators of virus activity in an area (Day and Lewis 1992, Reisen et al. 1994, Ramirez e al. 2018) and the use of enzyme-linked immunosorbent assays (ELISA) to test sentinel chicken serum is an effective tool (Johnson et al 2003).

As arbovirus infected female mosquitoes feed on sentinel chickens, the arbovirus is passed directly into the chicken blood from the mosquito saliva (Komar 2000). After an intrinsic incubation, antibodies develop in the sentinel chicken and can then be detected through serological assays. Though serological surveillance has an inherent delay (the need to wait for antibody development in the sentinel animal) and is less precise compared to other assays such as PCR, serology provides the benefit of providing location and time of transmission events. (Langevin et al. 2001). On the other hand, surveillance of arboviruses using mosquito pools can be difficult since mosquito infection rates are often extremely low in nature and does not represent transmission events (Day et al. 2015, Tang et al. 2020).

Some state health departments provide local mosquito control programs free or low-cost testing of sentinel samples. However, testing through large, centralized laboratories has its advantages and limitations. Advantages of the centralized laboratories include the comparability of results and the quality assurance of samples. Limitations of using centralized laboratories often include the delay in receiving results due to the large number of samples to be tested and the time required for shipping and receiving samples between programs (Day and Lewis 1992, Reisen et al. 1994, Sutkowska et al. 2021). The ability of mosquito control programs to receive their results in a timely manner helps maintain the relevance of those results for management and abatement decisions (Calisher et al. 1986, Johnson et al 2003). Outsourced laboratories often use a two-fold testing regimen which includes the initial "presumptive" result followed by confirmatory testing on presumptive positive samples. Though presumptive positive samples from outsourced laboratories may be provided within a few days, confirmatory results often take much longer. As districts often use confirmatory results to guide abatement decisions, a delay in confirmatory results being reported may negate the purpose of using sentinel samples as arboviruses would have been actively circulating before treatment measures have been deployed.

This study evaluated the effectiveness of a commercial competitive enzyme-linked immunosorbent assay kit (ID Screen West Nile Competition Multi Species; Innovative Diagnostics, Grabels, France – hereafter referred to as 'ID Screen') for in-house testing of sentinel chicken serum for WNV antibodies compared to the results from the traditionally utilized outsourced laboratory. As such, aliquoted subsamples from this study were also shipped to the outsourced laboratory for WNV antibody testing and confirmation. Sentinel chicken serum samples from this study were collected from the Anastasia Mosquito Control District (AMCD) and Lee County Mosquito Control District (LCMCD).

Sentinel chickens were bled weekly via a brachial or jugular venipuncture using a 3-mL syringe and a 25-gauge needle (Johnson et al 2003, FDOH 2021). Blood was transferred into 3.5-mL serum separator tubes (SST) and allowed to clot for at least 30-min and then centrifuged at >2,000 rpm for at least 10-min (Grasedieck et al. 2012). Serum was then removed from SSTs for testing and aliquots were shipped to the outsourced laboratory.

Sentinel chicken serum was tested in-house at both AMCD and LCMCD for WNV antibodies using the ID Screen kit according to manufacturer's instructions. In short, controls and samples were diluted with buffer and transferred into the kit provided pre-coated microwells of the ELISA plate. After a 90-min incubation at room temperature, the plates were washed three times (Model 1575 Immunowash Microplate Washer, Bio Rad, Hercules, CA) with approximately 300 µL of 1X wash solution. Diluted conjugate was then added to all microwells, and the plates were incubated for another 30-min at room temperature. After another round of washing, substrate solution was added to all microwells, and a final 15-min incubation was conducted in the dark at room temperature. After the final incubation, stop solution was added to all microwells, and the plates were read at 450 nm (iMark Microplate Absorbance Reader, Bio Rad, Hercules, CA). Optical density (OD) values were then converted into a signal-to-noise ratio (S/N%) using the following equation: ((ODsample / ODaverage negative control) * 100). Final results were reported as follows: S/N% of >50 were considered negative, >40 but ≤50 were considered doubtful, and ≤40 were considered positive (ID.Vet 2020).

Subsamples that were sent to the outsourced laboratory were initially screened (presumptive testing) using a hemagglutination inhibition assay (HAI). With the high volume of samples that come through the outsourced laboratory, this HAI assay was initially used to identify samples as being flavivirus antibody positive or not. If samples tested positive via the HAI assay, they were later confirmed positive for WNV (and/or SLEV) antibodies using an IgM ELISA assay and if needed a plaque reduction neutralization test (PRNT) (FDOH 2021). Assay protocols utilized by the outsourced laboratory are proprietary as they were provided to them by the Centers for Disease Control and Prevention.

During 2020 and 2021, a total of 311 sentinel chickens tested positive for antibodies (Table 1). Despite all 311 positive samples being tested by both the in-house and outsourced assays, only 300 (96.5%) were reported positive through in-house testing (WNV, ID Screen). The outsourced laboratory (WNV/SLEV, HAI) reported 212 (68.2%) positive samples (Table 1). Of the overall 311 antibody positive sentinel samples, 201 (64.3%) were positive via both in-house and outsourced testing, though not necessarily from the same week of testing.

Both the in-house ID Screen assay and the outsourced laboratory assay detected positive samples during the same sample week 182 times (90.6%, 182/201), however, the outsourced laboratory results took an average of 3.7 days longer to be reported in those instances (Table 1). The outsourced laboratory assay detected a positive sample prior to the week the in-house ID Screen assay was positive 17 times (8.5%, 17/201), averaging 9.7 days prior to the in-house ID Screen assay (Table 1). When taking into account the delay in results being reported from the outsourced laboratory, the results from these 17 instances were reported on average 6.0 days prior to the in-house assay results. The in-house ID Screen assay detected a positive prior to the week the outsourced laboratory assay was positive two times (1.0%, 2/201), averaging 46.0 days prior to the outsourced laboratory. When accounting for the delay, results from the in-house ID Screen assay were reported on average 49.5 days prior to the outsourced laboratory in these instances.

Table 1. Positive sentinel chickens from the Anastasia Mosquito Control District (AMCD) and Lee County Mosquito Control District (LCMCD) during 2020 and 2021 using the in-house commercial competitive ELISA kit and the hemagglutination inhibition assay results provided by the outsourced laboratory testing.

	2020		2021		Total
	AMCD	LCMCD	AMCD	LCMCD	
Number of positive birds	1	208	12	90	311
Positive birds from in-house testing	1	208	12	79	300
Positive birds from outsourced testing	1	110	12	89	212
Number of confirmed outsourced birds	1	71	4	41	117
Average days to receive initial outsourced results (range)	4 (4)	3.6 (3 to 4)	5.2 (4 to 11)	3.5 (3 to 4)	3.7
Average days to receive confirmed outsourced results (range)	60 (60)	10.3 (3 to 23)	8.5 (4 to 15)	13.0 (3 to 50)	11.6

Of the 212 presumptive antibody positive sentinel samples from the outsourced laboratory, 117 (55.2%) were later confirmed WNV positive via the PRNT. These confirmatory results were reported, on average, 11.6 days after samples were collected (Table 1).

In this study, the commercial ID Screen assay detected more of the positive samples compared to the outsourced laboratories presumptive HAI testing. A majority of the time, both testing methods detected a positive sample from the same sampling period; however, the outsourced laboratory had an inherent delay in reporting results, which is an important outcome of this study. The use of ELISAs for the detection of IgM antibodies has proven to have quicker turnaround times compared to HAI and PRNT assays from the outsourced laboratory (Calisher et al. 1986). The sooner these results can be reported to the control programs the sooner abatement efforts can be enacted, which directly relates to the overall effective control of potential WNV exposure. On that same note, confirmatory results from the outsourced laboratory took over three times as long to be reported when compared to the presumptive positive results. This delay in receiving confirmatory results from the outsourced laboratory may not be an issue if control programs base their abatement decisions on the presumptive results (WNV/SLEV).

The outsourced laboratory presumptive testing did detect positive samples from a sampling period prior to the in-house ID Screen assay at a higher rate (17 times verses 2 times). However, the magnitude between those differences was drastically greater when the in-house ID Screen assay detected a positive from a sampling period prior to the outsourced laboratory testing (average of 46.0 days prior compared to 9.7 days prior). This is something that must be considered by a program when deciding whether or not to use in-house testing methods for their sentinel program. An additional point of consideration is the associated cost of in-house testing. Unlike the free service often provided by outsourced public health laboratories, in-house testing is not free. There is an initial investment required to purchase the microplate washer and microplate reader as well as pipettes. Then there is the yearly cost of purchasing the commercial ELISA kits and consumables (pipette tips, etc.). For some control programs, this cost might be too great and thus the free service might outweigh the benefits provided through in-house testing.

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SUBMITTED ABSTRACTS OF THE 94TH ANNUAL MEETING

Hammock Beach Golf Resort & SPA, November 14-17, 2022

Subject Editor: Seth Gibson

Extrinsic incubation temperature impacts on infection and transmission of Mayaro virus in *Aedes aegypti* from Florida

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Mayaro virus (MAYV) is an emerging mosquito-borne arbovirus and public health concern. We evaluated the influence of temperature on *Aedes aegypti* responses to MAYV oral infection and transmission at two constant temperatures (20 °C and 30 °C). Infection of mosquito tissues (bodies and legs) and salivary secretions with MAYV was determined at 3, 9, 15, 21, and 27 days post infection. At both temperatures, we observed a trend of increase in the progression of MAYV infection and replication kinetics over time, followed by a decline during later periods. Peaks of MAYV infection, titer, and dissemination from the midgut were detected at 15 and 21 days post infection at 30 °C and 20 °C, respectively. Mosquitoes were able to transmit MAYV as early as day 3 at 30 °C, but MAYV was not detectable in salivary secretions until day 15 at 20 °C. Low rates of MAYV in salivary secretions collected from infected mosquitoes provided evidence supporting the notion that a substantial salivary gland barrier(s) in Florida *Ae. aegypti* can limit the risk of MAYV transmission. Our results provide insights into the effects of temperature and time on the progression of infection and replication of MAYV in *Ae. aegypti* vectors.

Rapid detection of arboviruses directly from mosquito saliva

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Mosquito-borne viruses continue to pose imminent threats to the health and economy of Florida. Florida's sentinel chicken program provides information on arbovirus activity to predict dynamics in arbovirus transmission risk. However, major disadvantages include the substantial time lags to antibody production and costs for maintaining flocks, which limit its usability as a tool for arbovirus surveillance. In this study, we developed a novel standardized platform to detect West Nile virus (WNV) and Dengue-1 virus (DENV) directly from the saliva of virus-infectious mosquitoes using loop-mediated isothermal amplification (LAMP) combined with a sucrose feeding stimulant. We first determined optimum sugar source and concentration to induce maximum mosquito feeding/salivation and found 40% aqueous sucrose resulted in the highest mean mass and proportional engorgement for mosquito females. We determined that premixed LAMP buffer combined with 40% aqueous sucrose was stable to detect arbovirus for an extended period (up to 48 hours) compared to premixed LAMP reagents without sucrose. Our results indicated that infectious female mosquitoes fed upon sweetened LAMP buffer (containing 40% sucrose) and deposited WNV and DENV in detectable quantities at rates equivalent to RT-qPCR. Importantly, no RNA extraction step was needed for our LAMP assays, so results could be interpreted within one hour of mosquitoes salivating into the LAMP reagents. Our newly developed optical image apparatus using a LED transilluminator visualized LAMP amplicons to discriminate between negative and positive samples. The sensitivity, simplicity, and rapidity of our novel LAMP platform could form the basis of an automated arbovirus detection system to minimize arbovirus surveillance time and effort of sample processing, the actual reaction step, and interpretation of results.

Potential transmission of *Francisella tularensis* between raptors by louse flies (Diptera: Hippoboscidae)

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Francisella tularensis is a pathogenic bacterium that can infect and cause disease in small mammals and birds. *F. tularensis* can be transmitted from direct contact of an infected organism, consumption / inhalation of bacteria or spores, or by arthropod vectors, including ticks and tabanids. Raptors are frequently diagnosed with tularemia disease, but the route of exposure is unknown as they are not often bitten by ticks or tabanids. Several species of birds are known to be parasitized by hippoboscid flies (louse flies); however, it is unknown whether these blood-feeding flies can transmit *F. tularensis.* In this study we explored potential modes of *F. tularensis* transmission within raptors and louse flies by using serological assays to determine the prevalence of *F. tularensis* in bird plasma and recently blood-fed flies. A direct ELISA was used to detect the presence of *F. tularensis* in louse flies and plasma of birds hosting louse flies. Indirect ELISAs for IgM and IgY against *F. tularensis* lipopolysaccharides (LPS) in raptors were used to detect the prevalence of antibodies in the plasma for an acute infection and to test for a broader history of exposure, respectively. Raptors that commonly fed upon rabbits and carrion had the highest prevalence of *F. tularensis*, presumably, due to direct transmission from infected prey. However, the prevalence in flies was significantly higher than in bird samples. Most of the birds that tested positive for *F. tularensis* in the direct and IgM indirect ELISA had shown that they had illness and symptoms when submitted to the rehabilitation center, corresponding to the birds that had tested positive. Overall, our findings suggest that louse flies may play a role in the transmission pathway of *F. tularensis* within raptors, although vector competence of these insects has not been confirmed.

Sentinel chicken program in Polk County

Hugo Ortiz Saavedra

Parks & Natural Resources, Polk County, Florida

Arbovirus surveillance remains one of the most important tools that mosquito control agencies in Florida have at their disposal. Arboviral transmission indices derived from surveillance from sentinel chickens, positive equines, positive humans, and positive mosquito pools provide indicators of local virus transmission. Using sentinel chicken seroconversion rates to estimate the frequency of mosquito transmission of arbovirus in a specific area, it is possible to obtain basic estimates of the risk of human arbovirus exposure. This can be used to measure the magnitude of overall risk and the use of proper alternatives for mosquito control. Since 1978, seven locations (chicken coops) in Polk County have been monitored. A total of 42 sentinel chickens, 6 birds from each location, are bled weekly by Polk County mosquito control staff. Blood samples are sent for testing to the laboratory at the Florida Department of Health in Hillsborough County Disease Control Unit in Tampa, Florida. Arbovirus surveillance in Polk County, Florida, consists of testing for endemic mosquito-borne viruses such as West Nile virus (WNV), Eastern equine encephalitis virus (EEEV), and St. Louis encephalitis virus (SLEV), as well as non-endemic viruses such as dengue virus (DENV), chikungunya virus (CHIKV), Zika virus (ZIKV), and California encephalitis group viruses (CEV). Malaria, a parasitic mosquito-borne disease, is also included. Arboviral activity for the last 10 years and experiences gained with the use of chickens as sentinels in Polk County will be presented.

Field flight activity of mosquitoes and *Culicoides* biting midges

Vilma Montenegro Castro and Nathan Burkett-Cadena

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Circadian rhythms govern daily temporal patterns of activity in terrestrial organisms. Increasing evidence demonstrates the importance of 24-hour circadian rhythms in vector-borne disease transmission. However, relatively little is known about the 24-hour circadian rhythms of mosquitoes and *Culicoides* biting midges (no-see-ums) and their implications for vector control. A simple system was developed to collect flying insects during the nighttime into five three-hour time period samples. The system consisted of a CDC miniature light trap with a wire mesh funnel at the outflow in place of a collection chamber. The funnel delivered trapped insects directly into individual compartments of a programmable rotating automatic pet feeder with six compartments, each filled with soapy water. Collections occurred five nights per week with nightly samples collected into three-hour periods between 1800 h to 0900 h. A large fraction (67%) of *Culicoides* biting midges were captured between 0300 h and 0600 h. Diverse mosquitoes, i.e., at least seven species from five genera, were collected. Sixty-four percent of *Deinocerites cancer* mosquitoes were collected between midnight and 0600 h, with up to 106 *De. cancer* collected nightly in this time period.

Insects with no medical importance (e.g., beetles, moths, and termites) were also sampled and consistency in their time of activity was observed. This study shows that relatively inexpensive products can be adapted to study circadian activity patterns of flying vector and nuisance arthropods, with implications for vector-borne disease transmission and control.

Environmental factors influencing *Aedes* container species distribution in St. Johns County, Florida

Kassidy Caride

Anastasia Mosquito Control District, St. Augustine, Florida

There have been observations that the distribution of *Aedes aegypti* in St. Johns County (SJC), Florida, is restricted to the coastal areas of the county. This led to the investigation of environmental factors influencing the distribution of urban *Aedes* container breeders in SJC. Three transects were selected running from the coastal part of SJC, west to the St. Johns River. The average distance from one transect to another was ~4 km. The trap points were selected along each transect at 7 different distances starting from the coast; each specific trap location was then determined by identifying residential locations suitable for container-inhabiting *Aedes* species. These locations were also prioritized by choosing premises along major roadways. Biogents Sentinel traps baited with BG lures and dry ice were placed once a week at each trap location for 10 weeks. For each trap location, three outdoor water-filled containers of both natural and artificial materials were sampled bi-weekly for water quality parameters. These parameters included salinity, pH, dissolved oxygen, and total dissolved solid levels; the parameters were used to understand environmental factors impacting *Aedes* container species distribution. The distribution of both *Ae. aegypti* and *Ae. albopictus* showed significant differences between different distances away from the coast. The numbers of *Ae. aegypti* were significantly higher up to 5 km from the coast, means ranging from 33.3 ± 6.48 (coastal) to 15.23 ± 4.47 (at 5 km). *Aedes albopictus* distribution was significantly lower at 3 km away from the coast than all other distances. Those distributions were correlated with the salinity, total dissolved solids, and pH levels measured in potential *Aedes* breeding containers at the same distances, in the same week, and 1 week before the trap collections. *Aedes aegypti* showed a significant positive correlation while *Ae. albopictus* showed a significant negative correlation. All three water parameters were correlated to each other with the salinity and total dissolved solids having the best correlation. None of the species showed a significant correlation with dissolved oxygen levels. The results justify the observed restricted distribution of *Ae. aegypti* in the coastal areas of the county while explaining the opposite behavior for *Ae. albopictus.* A laboratory study is underway to support these findings.

Evaluating BG-Counter performance in surveilling Collier County's diverse mosquito populations

Atom Rosales, Noe Pineda, Robert Straser, Keira Lucas, and Rebecca Heinig

Collier Mosquito Control District, Naples, Florida

The BG-Counter is a surveillance tool which is capable of remotely monitoring mosquito populations. Despite their use becoming more ubiquitous for mosquito control districts, there is a paucity of research on its performance in areas with high relative abundance and species diversity. Collier County is uniquely situated in southwest Florida between the Gulf of Mexico and the Everglades with over 50 recorded species. Mosquitoes were collected overnight by adding collection bags to BG-Sentinel 2 traps with BG-Counters at 7 locations across Collier County. Mosquitoes were sexed, counted, and identified to species. Non-mosquito arthropods were identified to family and categorized based on relative size. Total mosquitoes collected were compared against mosquitoes counted by the BG-Counter unit to evaluate its performance. Mosquito diversity, abundance, and counter accuracy will be discussed.

Surveillance trap report in St. Johns County, Florida, in 2022

Steven T. Smoleroff

Anastasia Mosquito Control District, St. Augustine, Florida

AMCD has an extensive surveillance program throughout St. Johns County (SJC). The purpose of our surveillance program is to monitor mosquito populations, mosquito-borne diseases, and environmental factors that influence mosquito populations. AMCD uses two primary trapping methods of CDC Light Traps (LT) baited with an octenol lure and BG 2 Sentinel traps baited with dry ice to monitor adult mosquito populations. AMCD has 41 CDC LT baited with octenol strips strategically spread throughout the county. These traps are traditionally first deployed in the spring during the month of April until November.

These data are used to determine and justify treatments by our aerial and ground field operations team. With the emergence of the Zika virus in the Americas in 2016, it prompted the need to monitor urban container *Aedes* species mosquitoes in SJC. AMCD places 12 BG 2 sentinel traps baited with dry ice and ovi cups to detect the presence of gravid *Aedes* mosquitoes. The BG 2 traps are deployed once a week year-round. The trap results have guided treatment efforts carried out in specific high-risk areas due to the yearly diverse population of tourists and travelers from around the world. This presentation will discuss AMCD's surveillance efforts for 2022.

Field evaluation of autocidal gravid ovitraps and SIRENIX traps against container-inhabiting mosquitoes in St. Augustine, Northeastern Florida

Dena Autry

Anastasia Mosquito Control District, St. Augustine, Florida

Mosquito control programs are utilizing cost-effective long-term autocidal traps targeting the gravid population of container-inhabiting and other mosquito species, with the aim of reducing vector populations and disease transmission risk. In this field study we directly compared the efficacy of the autocidal gravid ovitrap (AGO) and SIRENIX™ mosquito traps (SRX) in St. Augustine, Florida, to a control-only site that had no autocidal traps deployed. Total number of adult mosquitoes captured using BG traps baited with BG lure and dry ice were compared by pre-treatment (weeks 1-4) and post-treatment (weeks 4-8) to evaluate the attract-and-kill AGO and SRX efficacy at controlling *Aedes* and *Culex* species container mosquitoes. The post treatment numbers of both *Aedes aegypt*i and *Aedes albopictus* were increased in all three test areas. However, the mean percent changes were not significantly different across intervention and control sites. The numbers of *Culex quinquefasciatus* and total mosquitoes (all three species combined) were increased in the AGO area while those were decreased in the SRX trap area.

Evaluation systems for spatial repellents against ticks

Muhammad Farooq

Anastasia Mosquito Control District, St. Augustine, Florida

Spatial repellents are now becoming an important part of integrated vector management and are considered another vector-borne disease prevention tool as well as disease transmission breaking strategy. However, spatial repellents against ticks are still out of focus, in spite of the fact that humans are at a high risk of tick-borne diseases. Evaluations of spatial repellents are an important part of their development process and are key to determining the worthiness of the repellents. Evaluations against ticks are carried out either to assess efficacy of the AI, effectiveness of the delivery system, or a combination of both. These evaluations could be in the laboratory, under semi-field, or under field conditions. Many such techniques are discussed in this presentation. *D*-allethrin vapor generated by a personal mosquito repellent device was evaluated for its efficacy to spatially repel lone star ticks in a wind tunnel and in an olfactometer. Only adults were evaluated in the wind tunnel whereas adults and nymphs were evaluated in the olfactometer. The results of the wind tunnel study indicated some reduction in movement of ticks to the attractant, i.e., 31% compared to 49% in control. In the olfactometer tests, 87% of the adults moved away from repellent compared to 27% in control. These numbers were 43% and 20%, respectively, for nymphs.

Evaluation of silver nanoparticles as a control tool against adult mosquito vectors

Kai Blore

Anastasia Mosquito Control District, St. Augustine, Florida

Insecticides remain an integral component of mosquito control operations but sustained use of a limited number of active ingredients (AI) has led to the widespread development of resistance. New types of insecticides will be necessary in maintaining future control efficacy. Metal nanoparticles are a promising new tool which has demonstrated toxicity against mosquitoes across all life stages. In this study, nanoparticles were synthesized from silver nitrate ($AgNO_3$) using essential oils from different plants. Toxicity screening of these nanoparticles was then conducted via topical applications against adult female mosquitoes to assess their viability as standalone insecticides and for potential synergism with existing AI.

Impacts of differential mosquito control treatment regimens on insecticide susceptibility status of *Aedes aegypti* (Diptera: Culicidae)

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Aedes aegypti L. is an invasive mosquito species and notable vector of several pathogens in the USA. Their cryptic and anthropophilic nature puts this species in close association with humans, where they can also be a nuisance. Mosquito control programs are the front line of defense for protecting the community from nuisance-biting and disease. However, the occurrence and prevalence of insecticide resistance in mosquitoes is a well-documented phenomenon that directly impacts the efficacy of insecticide applications. In Florida specifically, widespread resistance in *Ae. aegypti* has created a need for operational strategies that combat and, ideally, reverse resistance. Laboratory studies and the association between fitness costs and insecticide resistance indicate that this reversion is possible under the right conditions. For a 2.5-year period, the impact of varying operational treatment regimens on insecticide resistance in *Ae. aegypti* is evaluated using *kdr* genotyping and the CDC bottle bioassay. In an organophosphate treatment area, a decrease in frequency of a double homozygous resistant genotype was observed. CDC bottle bioassays did not reveal any clear trends in the data to indicate a reversion to insecticide susceptibility. However, the changes in genotype could indicate the first step back to insecticide susceptibility. This study provides preliminary data that has implications for resistance management in mosquito control operations.

To fly or not to fly: an in-depth look at Florida mosquito control aerial capabilities

Steven T. Peper, Vindhya S. Aryaprema, and Whitney A. Qualls

Anastasia Mosquito Control District, St. Augustine, Florida

Effective integrated mosquito management (IMM) involves the implementation of a variety of control and educational approaches. Aerial application of larvicides and adulticides is a very effective tool, especially in programs that have vast territory or areas where it would be difficult for truck mounted applications, and is often considered the only practical approach to mosquito control. This study investigated the current aerial capabilities of mosquito control programs in the state of Florida. All 67 counties were attempted to be contacted and invited to participate in the on-line survey. Forty-one (61.2%) counties responded, of which 35 (85.4%) indicated they had aerial capabilities, either in-house or outsourced. Factors such as the use of aerial program, dedicated budget, number of pilots, mechanics, and aircraft, chemicals used, and so on will be discussed in this presentation.

New calibration calculators and swath characterization cloud applications

Leanne Lake

Valent BioSciences, LLC, Libertyville, Illinois

This presentation will review the new calibration calculators and swath characterization cloud applications available to end users on the Valent BioSciences webpage. A brief description of the interactive calibration calculators for all types of mosquito control applications platforms, types of equipment, and product formulations will be provided. The presentation will include a demonstration of using the tool. In addition, an overview of how to conduct aerial granular swath characterization analysis using the cloud-based app. This easy-to-use application allows applicators to conduct aerial swath analysis by simply uploading a single document.

Establishing evidence-based action thresholds for *Aedes*, *Culex*, and *Anopheles* species mosquito abundances in different operational environments

Vindhya S. Aryaprema

Anastasia Mosquito Control District, St. Augustine, Florida

AMCD is evaluating the development of action thresholds for *Aedes*, *Culex*, and *Anopheles* species mosquitoes in different operational environments. This presentation is focused on: (i) to identify published mosquito control action thresholds across the world and associated surveillance and implementation characteristics through a systematic literature review, and (ii) to develop statistical models using AMCD historical data on mosquito surveillance and associated climate data for determination of key variables and indices. The literature review of publications in the last decade (2010-2021) was conducted using two search engines, Google Scholar and PubMed Central, according to PRISMA guidelines. Of the 1,485 initial selections, only 87 were included in the final review. Three categories of publications were identified: (i) with originally generated thresholds (n=30), (ii) with statistical modeling procedures but no hard-defined thresholds (n=13), and (iii) with mentions of previously generated thresholds (n=44). Overall, the publications with "epidemiological thresholds" outnumbered those with "entomological thresholds". Most of the included publications were from Asia and those thresholds were targeted toward *Aedes* and dengue control. Mosquito counts (adult/larval) and climatic variables (temperature/rainfall) were the most used parameters in thresholds. The associated surveillance and implementation characteristics of the identified thresholds will be discussed in the presentation. The findings of the review (i) highlight data gaps and areas of focus to fill in the action threshold compartment of the integrated vector management toolbox, (ii) will help organize surveillance systems targeting development and implementation of action thresholds, and (iii) will make direct awareness towards already existing thresholds for programs lacking available resources for comprehensive surveillance systems. In the statistical modeling process, sixteen years of historical AMCD mosquito trap records (CDC light traps and BG traps) were mined and curated for analysis using a mixed bag of software tools. A timeline of epidemiological weeks (epiweek: Monday to Sunday) was developed to match collection days across trap systems and years. The basic climate parameters chosen for model development were temperature, rainfall, and relative humidity which were retrieved via online search tools. Two iterations of negative binomial regression models were constructed on 6 groups of mosquito species: (i) in form of continuous measurements, and (ii) with number of 'hot' or 'wet' or 'humid' days by exceedance of climate threshold values. Models were validated with predictive capability on testing data from the two recent surveillance seasons 2020 and 2021. Final significant climate predictors varied substantially between species groups. The most significant climate factors for the floodwater *Aedes* group, the dominant and operationally influential species group in the county, were either total precipitation or frequency of precipitation events at two to four weeks before trap collection week. Validation of models with testing data displayed limited predictive abilities. The models provide feedback for future modeling efforts of other mosquito control programs and guidance for deployed armed forces personnel with a need of mosquito control but lacking active surveillance programs.

Mobile insectaries: mosquitoes "on the go"

Victoria Hyrczyk

Clarke Mosquito Control, St. Charles, Illinois

Many mosquito control companies and programs have permanent insectaries to maintain mosquito colonies in a stable environment for mosquito control product trials and resistance research. As resistance monitoring becomes more critical to managing the useful life of mosquito control tools, field scientists and mosquito control districts see an increase in mosquito populations needed for laboratory testing and field trial work to assess and validate product performance. For field trial work in particular, the rearing and handling of mosquitos often need to happen "on the go," which is far more challenging and less productive than in permanent insectary settings. To counter these challenges, Clarke's biology and insectary teams have developed and vetted a set of guidelines for setting up mobile insectaries for in-field rearing of three common vector mosquito species: *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus*. This presentation will share the experience of the field science team responsible for creating this mobile insectary blueprint, highlight the challenges and best practices for developing mobile insectaries, and emphasize the importance of maintaining reliable local mosquito colonies for product efficacy studies.

Creating a good laboratory practice (GLP) program at Anastasia Mosquito Control District

Whitney A. Qualls

Anastasia Mosquito Control District, St. Augustine, Florida

Since 2019, Anastasia Mosquito Control District (AMCD) has worked on developing a good laboratory practice (GLP) program to evaluate public health pesticides. This process included hiring a GLP consultant to train AMCD on US Environmental Protection Agency (EPA) GLP Standards (40 CFR 160) and guide AMCD in the development of standard operating procedures (SOPs). A GLP Committee was formed at AMCD to develop working SOPs. In 2021, AMCD was contracted to conduct a GLP study for DNW Global, LLC, to evaluate a commercially available algaecide and bactericide. The GLP study assessed the efficacy of REXCU-S® ($CuSO_4 \cdot H_2O$) at various concentrations as a larvicide against mosquito species *Aedes aegypti, Anopheles quadrimaculatus*, and *Culex quinquefasciatus*. The study findings are intended to provide pesticide efficacy data for label claim purposes as part of the USEPA Pesticide Registration of REXCU-S. Based on USEPA guidelines for product performance and water quality requirements, a 95% mortality at a concentration of 1.3 ppm was the desired outcome of this study (OPPTS 810.3400 March 1998, 40 CFR § 141.80). This presentation will focus on the process of establishing the GLP program and the findings from AMCD's first GLP study.

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Journal of the Florida Mosquito Control Association

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FLORIDA MOSQUITO CONTROL ASSOCIATION

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