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ABSTRACT



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Plenary Session

PL-1 **A national public health strategy to reduce the burden of vector-borne diseases in the United State: current actions and future plans**

Lyle R. Petersen, LXP2@CDC.GOV, Charles Beard, Susanna Visser

The 2016-2017 Zika virus pandemic highlighted many deficiencies in the Nation's ability to respond to novel and existing vector-borne disease threats. In response, the CDC with five federal departments and the EPA developed the National Public Health Framework for the Prevention and Control of Vector-Borne Diseases in Humans, which detailed five strategic priorities for the Federal Government. Seventeen collaborating federal agencies are currently developing a national strategy to fulfill the goals outlined in the National Public Health Framework. In addition, with the aims of providing training at all levels, augmenting applied research relevant for vector control, and building surveillance and response capacities, CDC has provided funding to four new university-based Regional Centers of Excellence for Vector-Borne Diseases (CoEs), recipients of more than 30 targeted applied research contracts, 64 state and local health departments, and numerous partner organizations, including AMCA. New 2023 training and evaluation centers will extend the training reach of the COEs and complement the research of the COEs with a focus on prevention product and method evaluation. The newly developed Public Health Entomology for All program has been expanded and will provide even more opportunities for students from high school to post-graduate levels with the goal of diversifying the field of Public Health Entomology. These actions in aggregate should bring us closer to achieving the vision outlined in the National Public Health Framework to create a nation where vector-borne diseases no longer threaten public health.

Oral Presentations

Mosquito Lightning Symposium

1 **Making Mosquitoes Disappear on David Copperfield's Private Island in the Bahamas**

Ary Faraji, ary@slcmad.org

Diseases transmitted by mosquitoes are emerging across the globe in a broad range of urbanized, rural, and natural environments inhabited by their vector species. Because applications of insecticides remain the most effective, and often the only available tool to prevent or control mosquito-borne disease outbreaks, their use and scope continue to expand. However, the effects of multiple insecticide applications targeting adult mosquitoes on non-target insect communities remain poorly characterized. To remedy this knowledge gap, we conducted an evaluation of five aerial insecticide applications on insect communities in a natural environment near Salt Lake City, Utah. Employing a before-after-control-impact (BACI) approach, we assessed abundance and community composition changes over the study period utilizing Bayesian and community ecology analytical methods. We observed no discernible effects on most insect taxa, and there were no changes in the overall insect community composition. The abundance of Diptera, Coleoptera, and Hemiptera declined in control and treatment sites, Odonata increased over the period of the study, and Hymenoptera and Lepidoptera

remained similar suggesting seasonal trends rather than treatment effects. The only consistently detectable treatment effect was on non-biting midges (Diptera: Chironomidae), that are closely related to mosquitoes taxonomically and have similar body size and diel activity. Midge abundance declined by 79.9% (95% credible interval: 58.4- 91.9). Overall post-treatment abundance decline of 62.2% (95% credible interval: 22.5 – 87.8) was also detected for leafhoppers (Hemiptera: Cicadellidae), but, these declines were inconsistent and may be attributed to natural variability rather than the treatment effect. Treatment frequency, location, life-stage targeting, and application techniques may mitigate the effects of mosquito control on non-target insects to allow protecting human health while limiting environmental impacts.

2 Public Reaction to Social Media Alerts of Increasing WNV Activity

Dennis Walette, dennis@tangimosquito.org

In 2022, the Tangipahoa Mosquito Abatement District (TMAD) saw record levels of the number of mosquito pools testing positive for West Nile virus. As the numbers of positive pools increased each week, TMAD issued increasingly serious warnings of the potential risk for human infection. Press releases were sent to broadcast and print media outlets. Additionally, these alerts were posted on the TMAD Facebook page. This presentation will show the timeline of WNV activity and the alerts which were posted. Special note will be made of some of the more entertaining and memorable comments which were made by members of the public in response to our warnings. Some of these comments were completely dismissive of the potential threat - either because there was no danger, or that . Others said we were making all this up. Still others seemed to think it was some nefarious plot to wipe the public out.

3 Aedes papago of the Sonoran Desert

Lawrence Reeves, lereeves@ufl.edu

Aedes papago Zavortink is a unique and poorly known mosquito of the Sonoran Desert. The only member of its subgenus, *Abraedes*, *Aedes papago* is known only from the vicinity of Tucson, Arizona, and its larvae have never been found in nature – until the summer monsoon of 2022. *Aedes papago* was described in 1970 from a series of eggs collected with debris from an oak tree hole in Mendoza Canyon southwest of Tucson, and reared to the adult stage. Suspiciously, adult female *Aedes papago* are collected in the arid lower elevations between mountain ranges, far from the lower elevational limits of the Sky Islands' oak forests. In these areas, dominated by mesquite, palo verde, ocotillo, and cactus, water, and mosquito larval habitat, is exceptionally scarce. The larval habitat of *Aedes papago* in these dry habitats has been elusive. Here, we report new details on the bionomics of this mysterious desert mosquito.

4 Get ready for the BacDrop: A Cloudbased Larvicide Droplet Analysis Program for Wide Area Larvicide Applications

Katie Williams, katie.williams@valentbiosciences.com

WALS, a biorational application strategy for controlling container mosquito larvae in difficult to find or access habitats has been successfully utilized in various mosquito control programs worldwide. An important factor in ensuring effective WALS applications, similarly to adulticide

applications is droplet analysis. WALs requires a unique droplet size range in the extremely fine to fine, which can drift through areas such as neighborhood backyards where inaccessible containers may be abundant. To ensure that every application is effective, droplet characterizations are conducted prior to larvicide applications. However, up until now the droplet analysis was performed by a technical development specialist at Valent BioSciences. In hopes of making larvicide droplet analysis more streamlined and accessible for each mosquito control district that wishes to utilize the program, BacDrop will be available to the public and will be user controlled with individual log ins. Based on open-source software, BacDrop analyzes larvicide droplet data in less than 20 minutes.

5 **Exposure of *Culex quinquefasciatus* to abdominal sublethal adulticide disrupts oviposition behavior**

Robert Aldridge, robert.aldridge@usda.gov, Kenneth Linthicum

Mosquitoes exposed to sublethal concentrations of insecticides can have altered behavior. One behavior of interest is oviposition behavior. We exposed *Culex quinquefasciatus* females to sublethal doses of adulticides on the tip of their abdomen and observed they were unable to properly oviposit the raft of eggs leading to less survivorship of progeny. We highlight possible applications and means to disrupt oviposition behavior in *Cx. quinquefasciatus*.

6 **Get a little mushy**

Christopher Bibbs, chris@slcmad.org

In the vast sea of entomology, many would be familiar with Plant-Insect Interactions. This even includes the mosquito, with larval co-dependence on certain vegetative communities, harborage for adults, and nectar feeding. But what if I told you in the shadow of the giant, there grows another humidity-loving, darkness thriving, humble member of the once plant community? This talk will make you mushy for the mystifying mushrooms that make mosquitoes dance to a different tune. In a canvas of work across the scientific community (none of which has been generated by the author), we will explore how mesmerizing myco-mosquito interactions can be!

7 **Inside the Tiny and Fascinating World of Mosquito Sperm.**

Laura Harrington, lch27@cornell.edu

Did you ever think about mosquito sperm? Probably not. Most people do not even think about male mosquitoes since they do not suck blood and contribute to disease transmission. From ejaculate to storage and ultimately egg fertilization, sperm traverse a difficult, and at times, hostile journey to their final destination. After copulation, sperm briefly remain at the site of insemination until they are activated and swim to the entrance of the ducts that lead to the female's sperm storage organs (spermathecae). Within minutes, they travel up the narrow ducts to the spermathecae. The female nourishes sperm and maintains them in these permanent storage organs for her entire life. When she is ready, the female coordinates the release of sperm with ovulation, and the descending egg is fertilized. I will share some little-known facts about the biology of mosquito sperm during this journey that may change your perspective and, perhaps, your appreciation for the lowly male mosquito.

8 **Is it dominant? Is it recessive? Does it matter?**

Esther Cutshall, e_cutshall@u.pacific.edu

Are knockdown resistance (kdr) phenotypes recessive? This talk will give a brief overview on complete dominance and then look at the literature on kdr. Spoiler: the information from previous work is mixed. Then, a quick dive into recent work on *Culex tarsalis* looking at the relationship between number of kdr alleles and functional resistance in a bottle bioassay. Will we definitively answer the question on recessiveness? Probably not, but I hope it is interesting to think about!

9 **Journal Formatting: To Compromise or Not to Compromise**

Steve Peper, speper@amcdfi.org

Have you ever formatted a manuscript with specific journal guidelines just to have your advisor decide to submit to another journal? Or perhaps your initial submission was rejected. What next? Find a new journal and spend HOURS reformatting to the new journal guidelines. How many hours are lost each year with this tedious work? Here it is proposed that journals in similar fields unite and establish identical formatting guidelines. Will it be easy? No. Will it take compromise? Yes. But your grad students and staff will thank you!

10 **Novel Electrophysiological Methods for Observing Insecticide and Repellent Action in Mosquitoes**

Edmund Norris, Edmund.Norris@usda.gov

Resistance to currently utilized chemical insecticides and repellents represents a significant threat to public health. Better understanding the neurophysiological and musculoskeletal effects of currently available and candidate insecticides and repellents may help identify and develop novel chemical control tools. Here we highlight novel methods of recording nerve firing and muscle excitatory postsynaptic potentials in *Aedes aegypti*. In short, larval mosquitoes were dissected to expose the ventral nerve cord or lateral abdominal body-wall muscles to allow recordings using extracellular or intracellular microelectrodes. These methods represent new tools for probing the tissue-level effects of current and candidate chemical control tools on the market today. Not only can these methods be used to evaluate the potential of current and novel control chemistries to affect insect nervous system and muscles, they can also aid in elucidating the mechanisms of insecticides and repellents. This presentation will briefly describe these methods and discuss their potential for the field of public health vector control.

11 **The future of mosquito control: Oxitec self-limiting gene technology to control *Aedes aegypti***

Rajeev Vaidyanathan, rajeev.vaidyanathan@oxitec.com

Mosquito control technologies that we only imagined a generation ago are now being deployed and assessed routinely in the field. These technologies include Wolbachia-mediated cytoplasmic incompatibility, irradiation, dsRNA-mediated silencing, and precision guided Sterile Insect Technique (pgSIT) and CRISPR-mediated gene drives. Oxitec uses a self-limiting gene in *Aedes aegypti* that selects for the survival of male larvae and death of female larvae. Sex segregation is accomplished genetically rather than by human eye, a pupal sorter, or expensive image recognition software and laser-assisted ablation of adult females. This approach enables segregation of the sexes at the larval

stage in the field, ensuring only male mosquitoes are released. Hence, the cost and operational feasibility of this technology enables ease of use by mosquito control districts (MCDs), homeowners, and businesses, such as hotels and resorts. We will present results from Oxitec projects in Brazil and Florida that illustrate the efficacy and challenges of this technology.

12 Three Influential Papers

Lawrence Hribar, lhribar@keysmosquito.org

Three papers that influenced my development as a scientist will be mentioned. The first deals with preparation of visual aids for and delivery of an oral presentation at professional meetings. The second and third concern techniques for data analysis; statistics that might help people get more out of their data. "Guidelines For Giving A Truly Terrible Talk" is dated (much of it appropriate for the old 35mm slides) but some of it is still useful. This is more of a "use it as a bad example" article. "Kendall's "Tau" coefficient as an index of similarity in comparisons of plant or animal communities" presents a useful rank-order correlation that can be used to compare two or more biological communities. An online calculator is available and it will give a P value (good for publications). "The investigation of samples containing many species: II. Sample comparison" explains how to analyze data from samples that contain a lot of species but where some species greatly outnumber others.

13 Use of a pyriproxyfen-based larvicide in Chicago area catch basins

Justin Harbison, jharbison@luc.edu

In 2021, an assessment of a pyriproxifen-based larvicide, Sumilarv® 0.5G WSP, was performed in a small sample of 60 catch basins within the Chicago area North Shore Mosquito Abatement District (NSMAD). The percent of pupae successfully emerging from catch basins treated with a 1, 2, or 3 WSP-dose (25, 50, and 75 g respectively) was recorded and used to assess effective duration. Based on the results of this smaller experimental study in 2021, the 3 WSP-dose was applied operationally to more than 20,000 NSMAD catch basins in 2022. Back checks performed by NSMAD staff members during the 2022 season to more than 550 of these basins indicated that the applied dose was achieving the expected control within catch basins. A concurrent assessment of the methoprene-based extended-release Altosid XR Briquet®, a product used in Chicago area catch basins for over 30 years, was also performed in 2022 to provide a comparison of effective duration.

14 We put a jar on a stick and the internet went wild

Sarah Fink, sarahangle@franklincountyohio.gov, Zach Holbert-Watson

This presentation will detail how Franklin County Public Health came up with their idea for tick check stations in parks, implementation, and initial reception when a local acarologist posted on Twitter about the stations. Public health agencies and researchers reached out from across the country to enquire about our revolutionary idea to attach a jar to a stick to collect ticks. These tick check stations meet three objectives: 1. Prevent park visitors from relocating ticks from wild habitat to playground/picnic areas while removing them from their dogs, 2. Serve as a targeted, timely outreach and education tool where people are most likely to encounter ticks, 3. Provide passive surveillance data to our agency. We will review lessons learned from this pilot program, share surveillance results, and

provide tips to anyone who may be interested in implementing this low-cost tool in their area. Bonus content: cute dogs, amateur craftsmanship and tick pics.

15 **We Spray, What Sticks?**

Andrew Rivera, arivera@clarke.com, Tommy Pemrick

Ultra low volume cold aerosol spraying relies on creating a very large number of very small droplets so that the likelihood of impinging on mosquitoes is maximized. Previous research has investigated the size of droplets that impinge most efficiently as well as the location of the body where insecticides are most readily absorbed. High resolution photography allows for visual confirmation of past research, and an investigation of the influence of simulated cloud dynamics in a spray chamber. By taking a series of pictures at a fixed focal length and incrementally moving the subject closer to the lens, a composite image can be created that maintains sharp focus in all parts of an image. Imaging software can be used to count and size the droplets in various locations of the mosquito in a repeatable way as long as the focal length of the lens remains unchanged. High resolution visuals make the droplet requirements of ultra low volume sprays easy to communicate to new and seasoned mosquito control professionals alike.

16 **Lightning talk: Continent-wide patterns of mosquito diversity.**

Michael Reiskind, mhreiski@ncsu.edu

There are around 180 species of mosquitoes described from the continental US. In this lightning talk, I will describe the patterns of diversity, factors that might contribute to that diversity, and implications of diversity for the future of pathogen transmission and mosquito control, based upon data from the National Ecological Observatory Network (NEON). We have found diversity in the continental United States to follow predictable patterns of response to elevation and latitude, but this disappears when data from Alaska are included. We find geographic distance to be a good predictor of similarity between mosquito assemblages, while local land-use is not. We have also found that association between a known larval community--treehole species--is marginally supported by our data. We also describe the distribution and abundance of common pest and vector species that are important targets of mosquito control, as well as continent level phenological estimates of mosquito activity that might be useful in determining when to provide different types of control.

17 **Mmm, mmm good! Culex soup in the Chicago area**

Mark Clifton, mclifton@nsmad.com

Although they may be the most well-known, Campbell's Soup Company is not the only crafter of fine artisanal soups in the Midwest. The neighborhoods, cities and subterranean habitats of Chicagoland are becoming well-known for producing a unique local soup of their own; Culex soup. The recipe starts simple but rapidly becomes more complicated. To make the base of the soup, you first take the Culex pipiens cryptic species complex and hybridize Culex pipiens and Culex pipiens quinquefasciatus just north of the traditional "hybrid zone". One could stop there for a rather pedestrian take on this recipe. However, to create the piquant flavors this soup is really known for, a dash of the subterranean and autogenic Culex pipiens f. molestus is required. These three mosquitoes

are then thoroughly mixed in varying amounts throughout the area giving each neighborhood their own unique flavor profile. The triple disease threat of hybridized *Culex* soup is quite enough for most people. However, other areas like to spice things up like Emeril Lagasse (Bam!) by adding some *Culex* restuans, *Culex salinarius*, or *Culex erraticus* to the mix. Like deep dish pizza or Italian beef sandwiches, the complex mixture of mosquito flavors known as *Culex* soup is gaining recognition as another gourmet specialty of the Chicago area.

18 **Tires as Winter Dens for Tiger Mosquitoes**

Lyric Bartholomay, lbartholomay@wisc.edu, Katie Susong, Bradley Tucker

Asian tiger mosquitoes were detected in tire yards in Wisconsin in 2017. Was this cause for alarm and indication of imminent invasion and establishment, or an observation of a transient, seasonal invasion? Using lab-reared mosquitoes in induced diapause, some discarded unwanted tires, and temperature and humidity monitors, we have identified conditions that make tires into cozy overwintering dens for *Aedes albopictus* - even through the extreme cold winter conditions experienced in Wisconsin and Illinois, USA. Tires are well known as an oviposition site, supportive larval habitat, and mode of transport for this species. We show that tires provide a measure of insulation from cold temperatures. Additionally, we found that tires which are also covered in snow provide insulation for overwinter survival of *Aedes albopictus* eggs. These findings have important implications for successful establishment of this species, particularly in light of climate projections that suggest decreasing likelihood of deep snow cover.

19 **Of Mosquito Romance and Violins**

Michael Weber, michael.weber@biogents.com, Tim Ziemer

It has long been recognized that the sense of hearing plays a major role in mosquito mating. Male mosquitoes have exquisitely sensitive hearing to recognize the flight tones of females and pursue potential mates. Once in close proximity, the male will attempt to mate with the female. The ensuing interaction has been studied in great detail by several researchers who found that mating success is correlated with the male and the female shifting their frequencies to obtain a match (and, in fact, the female rejecting the male if it does not match). Surprisingly, in *Aedes aegypti* for example, this match does not occur at the fundamental wingbeat frequency but at a ratio of 3:2 between the female and male fundamentals, i.e. about 1200 Hz. The concept of "harmonic convergence" requires not only the ability for at least the female to perceive a frequency far above the previously established mosquito hearing range but also to determine that it is exactly three times her own wingbeat. Nonlinear mixing of flight tones has also been proposed, resulting in low frequencies well below the fundamental flight tones but in the established mosquito hearing range. In either case, how the female recognizes the "right" frequency to establish a match is as of yet uncertain. Using concepts from acoustic analysis and musicology, we present (augmented by sound and animations) a straightforward model of how male and female mosquitoes perceive their wingbeat duet. We also propose a robust mechanism by which females recognize a matching male (or reject him, which may have some relevance for SIT). Ultimately, deep understanding of mosquito acoustics may inform the development of novel mosquito control technologies.

20 **Children of the Corn: Using Drones to Combat the Demons**

Piper Kimball, piper@leateam.com, Bill Reynolds, Crystal Grippin, Mir Bear-Johnson, Hector Cardenas

The Southern San Joaquin Valley is known as a diverse agricultural region, not only for crops but also dairy production. Seed corn grows very well within the region with harvest occurring early in the late summer and early fall. For the Delta Mosquito and Vector Control district, harvest season coincides with the height of the West Nile virus (WNV) transmission cases found in birds, mosquitoes and potentially humans. The corn fields contribute greatly to the WNV issue since they provide a dense canopy and attractive food source for many birds, particular corvids and songbirds. To complicate matters, corn requires standing irrigation until harvest, and often the irrigated water is supplied from local dairy farm operations. This contributes to highly organic standing water from which the main mosquito disease vectors, *Culex quinquefasciatus*, *Culex tarsalis* and *Culex stigmatosoma* are attracted to as breeding habitat, with the birds serving as a convenient blood meal for the adult mosquitoes. The tall, dense corn vegetation further complicates vector control efforts as larval surveillance is hindered and conventional ground based applications of larvicides and adulticides are often ineffective in reducing the mosquito populations to acceptable levels. With all these factors considered, the use of unmanned aerial systems (UAS aka "drones") were implemented to assist in controlling the vector populations within the corn fields.

Legislative and Regulatory - Endangered Species Act and Mosquito Control Product Use Requirements: Know the Process Symposium I

21 Mosquito control and the Endangered Species Act: a National Marine Fisheries Service overview of addressing risks to aquatic species

David Baldwin, david.baldwin@noaa.gov, Tony Hawkes, Ryan DeWitt

A key requirement of the Endangered Species Act (ESA) is that a federal agency consult with the National Marine Fisheries Service (NMFS) when any action they take might affect an ESA-listed species managed by NMFS to insure that Federal actions are not likely to jeopardize the continued existence of listed species. The Environmental Protection Agency's (EPA) authorization of pesticide-containing products for the control of mosquitos is an action which has led to ESA-consultations with NMFS. This presentation will provide an overview of NMFS' approach to applying the ESA to address the risks posed to listed species by authorized uses of mosquito control products. The approach involves assessing the extent and magnitude of exposures to pesticides in order to assess the potential effects to listed species. In the case of mosquito control, this poses a challenge since use is not restricted to relatively defined sites (e.g. field of corn), but can occur over a wide area. Therefore, as part of ESA-consultations, NMFS has identified a need to better understand the extent of mosquito control use in order to manage the risk to ESA-listed aquatic species. NMFS is working with other agencies and organizations to develop the means to accomplish this goal.

22 Environmental Protection Agency update on pesticide use limitation areas

Susan Jennings, Jennings.Susan@epa.gov

Hear from the Environmental Protection Agency on new requirements and guidance for pesticide applicators and when those will go into effect. The Agency recently released timelines and methods for pesticide registration review. This presentation will cover what you need to know to stay in compliance with federal laws in 2023, specifically any new reporting requirements that will go into effect relating to endangered species protections.

23 Pesticide Mitigations to Address Endangered Species Concerns

Ashlea Frank, afrank@complianceservices.com

The registration and continued use of pesticides under the Federal Insecticide, Rodenticide, and Fungicide Act (FIFRA) is complicated by the requirement for US EPA's approved actions (i.e., registering a pesticide label) to comply with the Endangered Species Act (ESA). In 2022, EPA released a work plan and announced two pilots that explore processes and mitigations to help ensure the protection of endangered species from the use of pesticides. Examples of pesticide mitigations that are intended to reduce exposure to endangered species include general label statements, off-set requirements, application directions, wind and temperature advisories, environmental hazard statements, and geographic specific mitigations. When geographic specific mitigations are included on a pesticide label, they are commonly intended to address a species-specific concern and are accompanied by a spatial delineation identifying where mitigation is needed, also called a Pesticide Use Limitation Area (PULA). Using examples from recent registrations and actions, this presentation will discuss mosquitocide mitigations for ESA spanning from general label statements to specific mitigations, describe the PULA development process, and discuss ways to meet implementation challenges.

24 Bulletins Live! Two: What increased EPA requirements might mean for your district.

Edward Foley, foley@lcmcd.rg

The U.S. Environmental Protection Agency will soon be implementing new changes to define how mosquito and vector control programs operate with respect to endangered species. These new rules will require the use of their newly launched Bulletins Live! Two webpage in addition to increased reporting requirements. Agencies will also be required to work with their local national wildlife refuge to formulate a plan for treatments. While the implementation has yet to be fully communicated with local mosquito control programs, there are concerns that these increased regulations will be difficult for some districts to comply. At the Lee County Mosquito Control District, there are concerns the increased regulations will place undue liability on the part of the mosquito control district. With mosquito control districts already limited in their control abilities, any additional restrictions or liability will ultimately have deleterious effects to the service we can provide. Understanding these regulations now will help your operation be better prepared when the time comes.

25 Navigating a local US Fish and Wildlife Service consultation to comply with product labels

Mark Clifton, mclifton@nsmad.com

The Endangered Species act (ESA) was passed in 1972 to protect imperiled species as well as any habitat upon which they rely. Under Section 7 of the Endangered species act, Federal agencies are required to consult and cooperate with the USFWS and NMFS to ensure actions they take

further the goals of protecting endangered species. In 2018, a new consultation and evaluation process was utilized by the EPA to evaluate malathion, chlorpyrifos and diazinon. In 2021, the reregistration of malathion was completed by the EPA using this new interagency cooperation process. This reregistration is the first active ingredient used in mosquito control to be evaluated using input and data from the USFWS and NMFS. As a result of this new process, changes to the product labels for ULV materials are coming that require consultation with the Bulletins Live! Two website maintained by the EPA. Label requirements may also require a consultation with your regional US Fish and Wildlife Service office. This presentation will discuss how to conduct a FWS consultation in compliance with product labels as well as what resources are available to ensure a successful interaction.

Social Media and Mosquito Control Symposium

26 Searching social media posts in the "Sunshine"

Robin King, rking@cmcd.org

Transparency is a priority in Florida, thanks to the state's open government laws - known as "Government In The Sunshine" or "Sunshine Law." It provides the public with access to governmental records made as part of an agency's official business. These records include written documents, photographs, meeting minutes, and emails, but did you know all social media posts are included, too? As a special taxing district in southwest Florida, the Collier Mosquito Control District's records are subject to public review by request. A public records request arrived at the District focused on social media with the potential to require numerous hours of research. Specifically, the request was for any Facebook and Twitter post over a two-year period that included a list of key words. How much time would it take your organization to fulfill such a request? Sifting through thousands of posts may appear daunting at first glance, but learn how Collier Mosquito Control accomplished a monumental task in a matter of minutes.

27 Amplifying Your Voice on Social Media: Ideas from the Private Sector

Jeremy Hogan, jeremyh@celticchicago.com

Jeremy Hogan is the Vice President of Engagement for Celtic Chicago, a full-service marketing agency in the Chicagoland area. In his 16-year career, he has overseen the social media strategy for several clients in the B2B space. For more than a decade, he has partnered with insect control manufacturer Central Life Sciences across all markets, including its line of professional mosquito control solutions. While the social media objectives of private sector organizations may be different, the overall strategy and approach can still greatly benefit public sector social media efforts. Jeremy will share the four key phases of any successful social media campaign, including effective goal setting, strategic planning, implementation and evaluation. As he explains the steps he takes in each phase with his private sector clients, he will share several examples, findings, best practices and resources that can be utilized on social media by vector control and public health professionals.

28 What's Trending in Texas?

Jason Fritz, jason.fritz@wilco.org

Social media continues to be an important tool to share information quickly to a wide audience for many public and private agencies, including public health departments. Public health programs, including those focused on mosquito prevention and control, use various media platforms because they provide easy situational awareness to the public regarding health alerts, mosquito surveillance and control schedules, and other mosquito control activities to a large percentage of the population in an expeditious way for little to no costs. Texas is home to 5 mosquito control districts, and many other mosquito prevention and control programs operating within local municipal and state departments such as public health, environmental health, emergency management, and others. The Texas Mosquito Control Association (TMCA) has compiled various strategies from programs across the state regarding their social media presence in their communities and how they leverage different platforms to get their messages to the individuals who need them. The TMCA hopes to follow the social media messaging framework of the American Mosquito Control Association (AMCA) by highlighting stories from across the state and providing potential material for programs to utilize that may not have dedicated personnel for social media communications.

29 How to Leverage Agency Relations to Expand Social Media Reach and Engagement

Mary-Joy Coburn, mjcoburn@GLAmosquito.org

Social media is a powerful tool that informs and engages residents and stakeholders about our mosquito control message. Developing relationships with cities, partner agencies, and other relevant groups, and providing them with content takes time and experience that many mosquito abatement districts may not have – however doing so can pay dividends in community outreach and engagement with the public and stakeholders. Partner agencies, organizations, and respected community leaders can become an extension of a district and can reach more people that we can on our own. This presentation will provide tips from the Greater Los Angeles County Vector Control District (GLACVCD), as well as the American Mosquito Control Association (AMCA) on how to reach out to partner agencies and community leaders, create social media content and calendar / schedule to share, and discuss successes and challenges faced in the attempt to expand community outreach and resident engagement for mosquito control.

30 Increase Your Instagram Engagement 15 Seconds at a Time

Pablo Cabrera, pcabrera@sgvmosquito.org

There is no denying the dominance of social media in everyday life. In 2021, an estimated 48% of American adults said they get their news from social media. The explosion of TikTok sent social media platforms like Instagram into a frenzy to keep up with interactive short-form videos. Instagram released “Reels” on August 5, 2020 as a way to stay competitive against TikTok. According to Instagram, the platform currently has 1.4 billion active users worldwide, making it the fourth largest social media platform. Since releasing Reels, Instagram has increased its focus towards short-form videos and agencies should be aware of effective engagement strategies. For example, professional dermatologists have turned to Instagram Reels to promote literacy and provide accurate information to health consumers and prospective patients. By creating a strategy behind Instagram Reels, agencies can amplify their audience, help drive the narrative of their message, and be a resource of education. The San Gabriel Valley Mosquito and Vector Control District was an early adopter of Reels by posting its first Reel on August 18, 2020. Since then, the agency has created a strategy to amplify its reels by using

trending audio, creative transitions, geo-tagging, an engaging two-second opening, and reusing content. In 2021, the District posted 63 reels and averaged 1,586 views with 26 post engagements. But, in 2022, with a refined strategy, the District posted 59 posts and averaged 3,504 views with 72 post engagements. Another aspect that makes Instagram Reels valuable is that they get broadcasted to everyone and not just account followers. By content being shown to a wider audience, Reels allow agencies to be a part of the larger narrative and reach an audience that could be looking for education.

31 **Undoing the Creation of Doubt and Undermining on Social Media**

Jillian Meek, mosquitogeek@gmail.com

Eager to get started on social media, but worried about how to deal with the hostile or argumentative members of the social media community or even getting pushback from the boss to avoid the situation in the first place? Getting the buy-in from your citizens is important, and sometimes adverse posts and comments can create doubt, confusion, and chaos. Helping convey the understanding can be a big hurdle and remains an important task of public education in any integrated vector management plan, so let's get you ahead of the game and ready for anything! Undoing the negative perspective that is created on social media can sometimes be as simple as engagement, and other times may require some extra efforts. Being ready for whatever citizens throw at you helps the district and its staff on many levels. During this presentation we will provide some examples of common mosquito control pushback through comments, shares, group pages, and more all while giving some guidance and examples of how to combat that pushback.

32 **Twitter: the ins and outs of using the microblogging site to Tweet about mosquito control**

Michael Mut, mmut@miamidade.gov

This presentation will attempt to give attendees a robust look at Twitter, it's history and current functionality, and how it can be used to communicate with the public about mosquito control. I will provide detailed demographic information about the platform, step by step instructions on how to effectively tweet, and provide examples about how Miami-Dade County uses the site to talk to residents. I'll cover the different aspects, including protection tips, support for other County departments, communicating truck spray route information, and pitching stories to the media.

Part of an on-going series that began with "Social Media 101", "Social Media 202", and "Public Relations and Social Media", which have been presented at previous AMCA annual meetings (2019, 2021, and 2022). The natural extension of the series is this in-depth look at what is the presenter's favorite social media site. The hope is that users will take away insights on the medium, an expanded view of how to use it in their mosquito control programs. Presenter will be available after the symposia to discuss further and answer any questions.

Special District: Perspectives of Trustees and Commissioners Symposium I

33 **Ballot Measures and Funding for Special District to Face Financial Challenges and Service Needs**

John Bliss, john.bliss@sci-cg.com

Navigating the complex web of funding mechanisms to support mosquito and vector control agencies is extremely difficult - and even more difficult in this current challenging political environment. State laws strictly limits the ability of local agencies to generate revenue and a portfolio of approaches including ad-valorem property taxes; special taxes; Proposition 218-compliant benefit assessments; certain types of fees; and federal and state grants and loans, is often necessary. Of course, most vector control agencies continue to provide high levels of service with limited budgets while needing additional funding to address emerging reliance issues including climate change.

Attendees will receive a concise overview of viable funding mechanisms. The pros and cons, and administrative requirements of each mechanism will be presented, along with technical requirements, and political realities for each. The necessary planning and outreach to implement these mechanisms will be presented. Moreover, attendees will be presented with the recent and very significant changes in community outreach approaches including strategies to increase demographic inclusiveness in flood messaging.

Major topics include:

- Preparation, Planning and Financial Analysis
- Public Opinion Polling
- Revenue Mechanism Portfolio and Balloting
- Messaging and Community Outreach
- Case Studies: Winners and Losers

34 **Director/Manager Contract and Performance Evaluation**

Brian Tisdale, btisdale@rivco.org

One of the most important decisions a board will ever make is the hiring of a Director/Manager. Other than a district's general counsel and some rare additional exceptions for large special districts, the Director/Manager is the only individual the board hires and supervises. Another important consideration in hiring within special districts, the Director/Manager will report to the entire board. Having a unified board is important for the organization to move forward effectively. Clearly understanding your role as a policy maker will help in hiring that right person for day to day operation. A through understanding of the District's unique mission and the demographics is key to hiring the right person and developing a contract that meets all of the requirements of the organization and the community that it serves. Community involvement and scrutiny are factors that could influence the board decision on compensation and other benefits in the contract for the Director/Manager. Elected officials can be swayed by public opinion and sometimes it's hard to explain to public why an individual, using taxpayers dollars receive the types of benefits and compensation packages Within the contract, there should also be a complete understanding of how the Director/Manager is going to be evaluated.

The evaluation process is important to the organization and key to the organization moving forward. One major challenge with evaluations is the amount of contact trustees have with their Director/Manager. The trustees interaction will likely occur only during board meetings which could be quarterly or monthly. A tool such as a 360-degree performance appraisal that includes feedback from a supervisor, subordinates, colleagues and customers can help trustees with evaluation process. Open communications and a vision will create an environment in which contracting and evaluating the Director/Manager will result in an effectively run district.

35 Duties and Responsibilities of Manager or Director

John Smith, docmx8@gmail.com

Florida mosquito control district directors must meet specific requirements and perform high level managerial functions to be successful. They must meet qualifications and certifications mandated through Ch. 388 F.S. and 5E-13 F.A.C. These will be explained although most of the presentation will focus on describing the most common duties and responsibilities incumbent of a person serving in this capacity.

A list of duties and responsibilities was compiled by surveying 16 of the larger Florida mosquito control districts and programs. This was accomplished by asking each program to provide a copy of the Director's Position Description used for their agency. These were reviewed and synthesized into generic duties and responsibilities. Six areas of activities were identified to include: planning, human resources, resource allocation, Board activities, public relations, and other duties. Specific duties under each activity will be presented.

Although this presentation is from a Florida perspective, most of the duties should relate to directors and managers from other vector control programs.

36 Budgeting and financial management of a special district

Gary Goodman, gwgoodman@fightthebite.net

The fiduciary duty of the manager and the trustees of a special district is paramount to ensuring the future viability of the organization. Annual and long term decisions are based on the financial stability of the organization with the goal of maximizing the effectiveness and efficiency of the services provided to the public. This talk will discuss the components of creating a long term financial plan geared toward responding to the operational goals of the district.

37 State Sunshine Law and Its Implication to Florida Districts.

Trish Becker, Tbecker4amcd@protonmail.com

Anastasia Mosquito Control District (AMCD) of St. Augustine, Florida, was established in 1948. Then, it had three elected Commissioners and seventeen square miles to treat. Now, AMCD has five elected Board Commissioners, and it provides the entirety of St. Johns County with service- 609 square miles and a growing population of ~300,000. All Florida Special District Mosquito Commissioners operate under Florida Statute Chapter 388 and the Sunshine Law. At AMCD, a Commissioners term is four years, countywide, and nonpartisan. AMCD Commissioners receive a handbook that includes duties and responsibilities, Sunshine laws, benefits, detailed policies, and Robert's Rules for orderly meeting

procedures. The Board of Commissioners hires and evaluates the District Director, part-time lawyer, and a part-time CPA. In addition, the Board of Commissioners approves the millage rate and budgets and makes policies. The District Director implements the procedures and oversees the District's daily operations. The Board holds a monthly meeting on the second Thursday, except in November. The meetings are open to the public, and the District makes meeting announcements on its website and local newspaper in advance. In addition, each Board member chairs one of five committees, such as Planning, Financial, Education, Operations, and Applied Research. With everything that is done throughout Mosquito Boards, transparency is a must. The Sunshine Law must be followed and applied, so Board members cannot stack votes or pre-determine policy changes.

38 **District Governing Bodies in the United States – A Peek Behind the Curtain**

Dennis Walette, dennis@tangimosquito.org

Mosquito Abatement Districts (MADs, possibly called MCDs or MVCDs depending on where you are located) generally all have some sort of governing body—whether it might be a board of commissioners or trustees, or possibly some official within state or local government. This presentation will look at the results of a survey of representative control programs from around the country. Topics explored will include things such as how the members of these bodies are selected (whether they are appointed or elected); whether term limits exist; and whether the people that make up these boards are compensated in some way, and what form that compensation might take and what other benefits they might receive (insurance, retirement, travel, etc.). Also considered will be what differences might exist between Mosquito Abatement Districts and programs which are a department or agency within state or local government rather than a special taxing district. How these variables might affect a program's operational decisions will be discussed as well.

Student Competition I

39 **Chemical Constituents and Larvicidal efficacy of seven Medicinal plant Leaf Extracts against Dengue Vector *Aedes aegypti* L (Diptera : Culicidae)**

Shah Fahad, shahfahad@bs.qau.edu.pk

The mosquito-borne flaviviruses are causing major diseases in humans every year across the globe, resulting in a significant burden of morbidity and mortality. Dengue is the most common flavivirus mosquito-borne disease predominantly transmitted by the *Aedes (Stegomyia) aegypti* mosquito. It is one of the fastest-growing global infectious diseases, with 100–400 million new infections a year. Phytochemicals are advantageous in vector control strategies due to their target-specificity, eco-friendly, and no development of resistance, higher acceptability and suitability for rural areas. The current study was carried out to assess the role of larvicidal activities of seven Pakistan native medicinal plants viz. *Albizia lebbek*, *C. fistula*, *D. sisso*, *F. palmate*, *M. azedarach*, *Z. mauritiana* and *Z. namularia* against the 1st to 4th instar dengue vector *Ae. aegypti* larvae reared in entomology laboratory, Quaid I Azam University Islamabad, Pakistan. The experimented plants extracts (80 ppm to 400 ppm (multiple of 80)) were taken in 300 ml plastic containers, added 200 ml distilled water and 25 larvae, fed with dog biscuits. Each experiment was setup in triplicates along with control. Among the experimented plants *M. azedarach* found to be the most effective. The *M. azedarach* showed 96% mortality of 1st instar larvae

at 400 ppm after 72 hrs. showing LC50 (114.179) and LC90 (433.425) with Chi-Square value of 33.3039, DF:4 and P = 0.000. The morphology of dead larvae observed under microscopic showed adverse effects of plant extracts indicating shrinkage and damaged comb scale and siphon. The plants were comparatively analyzed for their biochemical constituents using UV, FTIR, GCMS. The results obtained from this study showed a potential toxicity of *M. azedarach* extracts in the control of *Ae. aegypti* mosquito larvae. This is a new eco-friendly approach which can be used for vector control. Therefore, this study provides first report on the larvicidal activities of seven plant extracts against larvae of *Ae. aegypti* mosquito from Pakistan.

Key words: Larvicidal bioassay, *Aedes aegypti*, mortality, plant extracts, concentration, probit analysis

40 **Time course analysis of pyrethroid genetic response in *Aedes aegypti* from California.**

Lindsey Mack, lkmack@ucdavis.edu, Geoffrey Attardo

The arbovirus vector, *Aedes aegypti*, has colonized California over the past decade. Their success is facilitated by resistance to pyrethroids, the primary public health insecticide. To define the genetic responses assisting insecticide exposure recovery, we performed a high-throughput time course analysis of gene expression following sub-lethal exposure to permethrin. Results reveal enrichment of genes mediating energy metabolism and oxidative stress responses. Inhibition of these responses could be a potential target for pyrethroid synergist development.

41 **Fine-scale associations between land cover and the community composition of *Aedes* (Diptera: Culicidae) mosquitoes**

Corey Day, cday11@vols.utk.edu, Rebecca Trout Fryxell

The principal La Crosse virus (LACV) vector, *Aedes triseriatus*, is associated with forested habitats and may be outcompeted by accessory vectors in urban areas. We used standardized oviposition cups to sample LACV vectors along an urbanization gradient in 2021 and 2022 and found that the relative abundance of *Ae. triseriatus* increased with site-level canopy cover. Spatial heterogeneity in LACV transmission risk may be related to land cover and associated vector community compositions.

42 **Bunyavirus Detection in Invasive *Aedes* Mosquito Species from Air Force Installations, 2018 - 2020**

Joshua Lassen, lassen.2@wright.edu, Leah Colton

Aedes albopictus and *Aedes japonicus* are introduced mosquito species that can transmit several North American arboviruses. However, these species contributions to local amplification and transmission cycles are poorly understood. *Aedes triseriatus*, in contrast, are native and the primary enzootic vector of La Crosse virus (LACV), the leading cause of pediatric arboviral encephalitis in the U.S.

To investigate their role in arbovirus transmission, we reserved 2,634 mosquitoes taxonomically identified as *Ae. albopictus* (92%), *Ae. japonicus* (3%) and *Ae. triseriatus* (5%), submitted from 29 Air Force installations (2018 – 2020). Most were captured in 3 states: Virginia (39%), Florida (26%) and

North Carolina (6%). We pooled mosquitoes by species (≤ 10 /pool) and tested them with either TaqMan or SYBR green assays for La Crosse, California serogroup (CAL) and Jamestown Canyon (JCV) viruses.

Most pools were negative for all viruses tested, but some samples generated low positives for LACV (15), CAL (12) and JCV (2). We expected the CAL assay to confirm LACV and JCV findings but interestingly, results were not confirmed for most of these. Most LACV positives came from Virginia (4) and Florida (4). The Florida findings were unexpected as LACV human cases are rarely reported there. Among CAL positives, 4 came from Virginia: one *Ae. triseriatus* pool from June 2019 and 3 *Ae. albopictus* pools from August 2020, all from different locations on the installation. The 2 JCV positives were from Mississippi and Georgia. This appears to be a novel finding; we found no reports of JCV in mosquitoes from these states.

Results suggest circulation of bunyavirus variants on several installations. Given that sample sizes per installation were generally small, and 2020 surveillance was reduced due to COVID-19, future targeted sampling at specific installations can validate whether these invasive mosquitoes are infected with any bunyaviruses.

43 **Evaluating vector mosquito occurrence in residential rain barrels in Champaign County, IL**

Rebecca Cloud, rcloud3@illinois.edu, Andrew Mackay, Catherine Wangen, Brian Allan

Rain barrels are green infrastructure tools used to conserve rainwater for outdoor use. Several species of vector mosquitoes complete juvenile development in artificial containers, with rain barrels serving as a potential source in residential neighborhoods. We conducted periodic inspections of rain barrels located at 54 households to identify conditions associated with mosquito positivity, and found over 50% of households contained at least one mosquito-positive rain barrel. These findings will inform mosquito prevention in green infrastructure.

44 **Microplastic ingestion alters the composition of *Aedes* mosquito species microbiota.**

Carla-Cristina Edwards, carlaedw@ttu.edu, Gabriella McConnel, Corey Brelsfoard, Jaclyn Canas-Carrell

To date, no studies have examined the effects of microplastic ingestion by container inhabiting mosquitoes. Here we show when *Aedes aegypti* and *Ae. albopictus* larvae ingest microplastics, there is resulting dysbiosis of their microbiota. Understanding the effects of microplastics on microbiota composition will be crucial for predicting and effectively mitigating the effects of microplastics exposure in mosquitoes and other insects.

45 **Expanded phylogeographic analysis of the Asian longhorned tick (*Haemaphysalis longicornis*) using mitogenomics**

Zoe Narvaez, zen5@connect.rutgers.edu, Andrea Egizi, Dana Price

The Asian longhorned tick (*Haemaphysalis longicornis*) is an established invasive tick vector in the United States. To better understand the invasion and population dynamics of this species, we applied mitogenomic sequencing and phylogeographic analysis of *H. longicornis* collected from native and expanded ranges. We highlight three potential introductions to the US, with source

populations of each identified in Korea and Japan. These findings provide insight into potential routes of entry for this tick.

46 **Assessing permethrin resistance for *Culex tarsalis* in Southern California**

Julie Tsecouras, jtsec001@ucr.edu, Tara Thiemann, Kim Hung, Jennifer Henke, Alec Gerry

The most common target site insensitivity in *Culex tarsalis* is a knockdown resistance (kdr) mutation. *Cx. tarsalis* were collected from five southern California locations with varying environmental and habitat characteristics to determine prevalence of permethrin resistance. Permethrin resistance was evaluated through CDC bottle bioassays to assess a resistance phenotype and by qPCR/sequencing to detect the presence of a kdr mutation (genotype). Phenotypic and genotypic resistance was detected and varied by location.

Factors Allowing for the Transmission of Vector-Borne Viruses: Vector Competence, Vertebrate Host Competence, and Environmental Factors Symposium

47 **What makes a particular species of mosquito a competent vector?**

Rebekah Kading, rebekah.kading@colostate.edu

Mosquitoes are responsible for the transmission of numerous human-pathogenic viruses and parasites of global health significance. The term “vector competence” describes the intrinsic ability of an arthropod vector to transmit an infectious agent. While transmission events by blood-feeding mosquitoes may seem straightforward conceptually, the mosquito itself presents a complex and hostile environment through which the infectious agent must transit to ensure the propagation, sometimes development, and transmission of itself to the next host. Viruses imbibed in an infectious blood meal must pass into and out of the mosquito midgut, traffic through the body cavity or hemocoel, invade the salivary glands, and be expelled with the saliva when the vector takes a subsequent blood meal. Parasitic nematodes also utilize different organs as developmental sites, such as the Malpighian tubules or thoracic flight muscles. For both viruses and parasites, physical, cellular, microbial, and immunological barriers are encountered during this journey, which is also influenced by the genetic background of the mosquito vector as well as environmental conditions. This presentation will provide an overview of the current state of the field in understanding the mosquito-specific factors that underpin mosquito vector competence, and ultimately what makes a particular mosquito species a competent vector.

48 **Lethal and sublethal effects of insecticides: Within species variability of mosquito vector competence**

Barry Alto, bwalto@ufl.edu, Abdullah Alomar, Kauara Campos

The application of insecticides may include both lethal and sublethal effects, and associated development of insecticide resistance. Mosquitoes with phenotypes that exhibit insecticide

resistance or experience sublethal effects may be associated with altered vector competence for arboviruses. Here, we present evidence for within species variability in vector competence associated with exposure to insecticides among invasive *Stegomyia* mosquitoes.

49

What makes a particular vertebrate an important amplifying host?

Jefferson Vaughan, jefferson.vaughan@und.edu

In nature, mosquito-borne arboviruses cycle from viremic vertebrate hosts to appropriate mosquito vectors and back again to immunologically naïve vertebrate hosts. But there is a certain amount of specificity to these cycles. Just as not every mosquito species can transmit every arbovirus (thankfully), not every vertebrate species can serve as good amplifying hosts for every arbovirus. There are several behavioral and population-based attributes of vertebrate species that can contribute to their status as amplifying hosts. These include such things as; host attractiveness to mosquitoes, defensive behavior against mosquito attack, the relative abundance of hosts, and the reproductive turnover of hosts (i.e., recruitment of new, non-immune individuals). But underlying all of this is the innate susceptibility of the vertebrate to an arbovirus. This has traditionally been measured as the intensity and duration of viremia (i.e., virus concentration in the blood) that a host species experiences upon infection. For arbovirus transmission cycles involving multiple host species (e.g., West Nile virus), the relative contribution of a host species traditionally has been inferred from its viremia – that is, species developing high viremias are more important amplifying hosts than species developing low viremia. Intuitively, this makes sense and the idea that higher viremia invariably results in higher infectiousness to mosquitoes has led to a concept known as “host competency”. But recent studies have suggested that host infectiousness to mosquitoes may not always be linked just to host viremia. This presentation will examine our current understanding of “host competency” and will discuss recent findings that may improve that understanding.

50

How the presence of other organisms in a viremic vertebrate host may affect the ability of a mosquito feeding on that host to be able to transmit that virus.

Michael Turell, mbturell@gmail.com

In order for a mosquito to be able to transmit a virus, it must feed on a viremic animal, and the virus must infect the midgut, escape into the hemocoel, enter the salivary glands, and be secreted into the saliva before that mosquito is able to transmit that virus by bite. Some mosquitoes have midguts that are not susceptible to virus infection, or even if they are, the time from virus ingestion to when they can transmit virus by bite may be over two ovarian cycles. However, in nature, vertebrates often are infected with more than one pathogen (e.g., filarial infection and an arbovirus or malaria and an arbovirus). Because both filaria and malaria infections can persist for long periods of time, animals infected with either of these pathogens, if they become infected with a virus, may allow mosquitoes to ingest both that pathogen and the virus concurrently. Because microfilariae penetrate the midgut almost immediately after a bloodmeal, they may allow a virus to bypass both midgut infection and midgut escape and infect the hemocoel within hours of ingestion. This would not only allow for a much higher dissemination rate but would also greatly shorten the extrinsic incubation period. Similarly, as malaria sporozoites penetrate a salivary gland, they may allow a virus to bypass a salivary gland barrier. However, as with viruses and mosquitoes, there appears to be a species-specific interaction between viruses and these other pathogens. This presentation will illustrate how the

presence of these other pathogens can significantly affect the ability of a mosquito to transmit a pathogen and even turn an essentially incompetent mosquito into a competent vector.

51 **Unraveling the complex influence of environmental temperature on vectorial capacity of mosquitoes for arboviruses**

Alexander Ciota, alexander.ciota@health.ny.gov

The intergovernmental panel on climate change (IPCC) has reported a 1.1°C increase in global temperatures since the mid-1800s, with accelerating warming noted in each of the last four decades. Although the impacts of climate change on public health are far-reaching, the biology and ecology of mosquitoes and the pathogens they carry are uniquely influenced by environmental variability. Given that mosquitoes are ectothermic organisms, temperature shifts directly influence numerous aspects of vectorial capacity, the overall transmission potential of a population. Increasing temperatures generally increase arbovirus replication, accelerating extrinsic incubation periods and increasing overall vector competence. Mosquito development is additionally accelerated at increased temperature. The consequence of decreased development time is presumably increased population size, but increased temperature can also increase immature and adult mortality, altered blood feeding, differential mating success and altered hatch rates. Findings from studies evaluating the effects of temperature on adult longevity generally indicate decreased longevity at increased temperatures, to some extent countering increases in transmissibility at higher temperatures resulting from increased development rates and vector competence. Despite the fact most experimental studies have not fully evaluated thermal limits, these relationships are unimodal, implying that how temperature shifts effect arbovirus transmission is dependent on the current conditions of any given population. In addition, while generic trends exist, variability in the relationship between arbovirus transmission and temperature among mosquito species and populations is well documented, implying that distinct mosquito genetics, viral genetics, transcriptomics, immunity and/or microbiota can influence vectorial capacity in ways that can either reinforce or supersede the independent influence of temperature. Lastly, given that transmission is dependent of highly dynamic and evolving biological systems, we must consider how future temperatures could interact with future arbovirus strains and vector populations. Defining these complex relationships is critical to predicting how environmental warming could have region-specific effects on vectorial capacity.

52 **The effect of rainfall on the ability of mosquitoes to transmit a virus or parasite and disease occurrence**

Kenneth Linthicum, kenneth.linthicum@usda.gov, Assaf Anyamba, Seth Gibson

We will discuss the impact of rainfall on the ability of mosquitoes to transmit vector borne diseases and demonstrate that global climate teleconnections can be used to anticipate and forecast, in the case of Rift Valley fever, epidemics and epizootics. In this context we will examine significant worldwide weather anomalies that have affected vector-borne disease outbreaks. Utilizing vegetation index data from NASA's satellite based Moderate Resolution Imaging Spectroradiometer (MODIS) to map the magnitude and extent of these anomalies for diverse regions including the continental United States, Russia, East Africa, Southern Africa, and Australia we demonstrate that shifts in precipitation have significant impacts on vegetation patterns with consequences for public health. Weather extremes have resulted in excessive rainfall and flooding as well as severe drought. These

extremes have created exceptional conditions for extensive mosquito-borne disease outbreaks of dengue, Rift Valley fever, Murray Valley encephalitis, West Nile virus and malaria. The prognosis for vector-borne disease transmission in a changing climate will be discussed.

Latin American Student Competition

53 Polymorphisms in the vgsc of Aedes aegypti and Aedes albopictus from Mexico

Perla Cecilia Martínez, ceciliamschz@gmail.com, Mitzi Garcia-Bernal, Beatriz Lopez-Monroy, Andre Castro-Bautista, Gustavo Ponce-Garcia, Adriana E. Flores-Suarez

The effectiveness of the products used to control the arbovirus vector mosquitoes has been reduced, which is associated with the point mutations (kdr) present in the voltage gated sodium channel. To date, the emergence of new substitutions in vgsc of Aedes aegypti and Aedes albopictus has been reported in other countries, for which reason the presence / absence of these in Mexican mosquito populations was determined in the present study.

54 Polymorphisms in voltage gated sodium channel (vgsc) gene and its association with resistance to pyrethroid insecticides in Aedes aegypti (L.) (Diptera: Culicidae) populations from Mexico

Andre Castro-Bautista, andrecastrobautista@gmail.com, Perla Cecilia Martínez, Beatriz Lopez-Monroy, Gustavo Ponce-Garcia, Jesus A. Davila-Barboza, Ildefonso Fernández-Salas, Adriana E. Flores-Suarez

Widespread use of pyrethroid insecticides to control the main dengue vector Ae. aegypti, has caused selection pressure in a molecular mechanism of resistance, point mutations in the vgsc gene, named kdr for the phenotypic effect of knockdown resistance in individuals who have them. In this study, we evaluated the relationship between the frequency of kdr mutations in the vgsc gene and the response after exposure to bifenthrin and deltamethrin in Mexican populations of Ae. aegypti.

55 Aedes aegypti control using synthetic double-stranded RNA

Mariana Lizbeth Jiménez-Martínez, mariana.jimenez80@gmail.com, María de Lourdes Ramírez-Ahuja, Gerardo de Jesús Trujillo-Rodríguez, Iram Pablo Rodriguez-Sanchez

The mosquito Ae. aegypti is the vector that transmits the dengue virus and other important pathogens in America. Interfering RNAs have been shown to be mediators of gene expression. Silencing RNA is a conserved mechanism in nature in which small double-stranded RNA (dsRNA) regulates gene translation.

The objective of our study was to evaluate the effect of inhibiting the translation of exon 5 of the DSX gene using antisense RNA (dsRNA).

56 Comparison of Aedes aegypti populations resistant to pyrethroid insecticides against those formulated in vitro

Gerardo de Jesús Trujillo-Rodríguez, entogerry36@gmail.com, María de Lourdes Ramírez-Ahuja, Mariana Lizbeth Jiménez-Martínez, Iram Pablo Rodriguez-Sanchez

The *Ae. aegypti* mosquito is the main vector in America. There are several control programs, many of which are ineffective, and therefore, new control alternatives need to be developed. Resistance to insecticides is one problem, for which it is necessary to develop new methods of monitoring resistance. Our objective was to test 3 populations resistant to pyrethroids against a formulation composed of imidacloprid and pralethrin to compare resistance between pure and formulated insecticides

57 **Population biocontrol of *Aedes aegypti* with simple synthetic RNA.**

María de Lourdes Ramírez-Ahuja, lulu.ahuja@hotmail.com, Gerardo de Jesús Trujillo-Rodríguez, Mariana Lizbeth Jiménez-Martínez, Iram Pablo Rodriguez-Sanchez

The mosquito *Ae. aegypti* is the vector that transmits the dengue virus and other important pathogens in America. Interfering RNAs have been shown to be mediators of gene expression. Silencing RNA is a conserved mechanism in nature in which small double-stranded RNA (dsRNA) regulates gene translation. The objective of our study was to evaluate the effect of inhibiting the translation of exon 5 of the *DSX* gene using antisense RNA (SiRNA).

Legislative and Regulatory - Endangered Species Act and Mosquito Control Product Use Requirements: Know the Process Symposium II

58 **Endangered Species and Registration Review for Mosquitocides**

Paul Whatling, paul.whatling@fmc.com

After many years of litigation against the United States (U.S.) Environmental Protection Agency (EPA), the U.S. Fish & Wildlife Service (FWS), and the National Marine Fisheries Service (NMFS), case law has clearly established that EPA's authorization to register or reregister pesticides is an agency action that requires review under the U.S. Endangered Species Act (ESA). The ESA requires that agency actions do not jeopardize the continued existence of an endangered species, or adversely modify its critical habitat. EPA's action of registering or reregistering insecticides to control adult and larval mosquitoes is an action that requires ESA review. All insecticides used for mosquito control will be evaluated under the ESA; however, thus far only malathion has been fully evaluated. During the ESA consultation on malathion, innovative thinking was needed from AMCA, the pesticide registrant, and the Services to develop measures that allowed public health mosquito control to continue while providing protection to endangered species. Lessons learned from the ESA evaluation on malathion relevant to all current use mosquito adulticides and implications on mosquito control operations will be discussed.

59 **Process of Making Refinements to the Models used by the Environmental Protection Agency when Public Health Pesticides are Registered for Area Wide Ultra Low Volume Adult Mosquito Control**

David Brown, Dabrownsoj@gmail.com

The Environmental Protection Agency (EPA) uses models to help evaluate risk for human health and potential impacts to Endangered Species when they are registering /reregistering pesticides. It is an inexpensive and efficient method for EPA to evaluate the thousands of pesticides currently on the market or being considered for new registration. However, the current models being used by EPA were initially developed for agricultural and forestry applications, and because of the difference in application methodology used for adult mosquito control the potential risks to human health or Endangered Species within a spray area are often grossly overstated. This paper will discuss the process used by the American Mosquito Control Association Legislative and Regulatory Committee (which includes, but was not limited to, input from the Chemical Control Sub-Committee, the Endangered Species Act Sub-Committee The Washington Day Sub-Committee, and the Federal Funding Sub-Committee) to obtain funding, perform field trials, and collect empirical data for submission to EPA to refine the current models to better evaluate any potential affects from Area-Wide Ultra Low Volume Adult Mosquito Control Applications

60 **The Impact of New Pesticide Regulations to Protect Endangered Species**

Daniel Markowski

There will soon be regulatory changes affecting many of our mosquito control programs. As the Malathion registration review continues, EPA and the Services are close to finalizing the Endangered Species protections that will include mitigations for at least 22 species of concern. Label changes will direct end users to Bulletins Live! Two where we'll find the usage limitations for our operations. Currently, there are 2 key mitigations that will be required: If we cannot avoid the application of Malathion and likely other mosquitocides) to Pesticide Use Limitation Areas (PULAs) defined for each species of concern, users will have to consult with the local Fish and Wildlife Service offices to determine appropriate measures to ensure the proposed application is likely to have no more than minor effects on the species. Additionally, we will in all likelihood have to annually report to EPA basic usage information such as the number of applications, location of each application identified by latitudinal and longitudinal coordinates, and area of each treatment for all of our mosquitocides. The details are still being finalized; however, we should prepare now for these regulations likely coming in 2023.

61 **RESPECT Plan- Report of Endangered Species and Pollinator Evaluation of Chemical Treatment-A decision making template for non-target assessment in mosquito management**

Dan Killingsworth, dan.killingsworth@pest.com

Mosquito management entities must remain vigilant in consideration of protecting non-target animals when treating. Evaluating organisms of concern by species, protection status, and habitat range are paramount to success. Public health mosquito control providers responsible for the entirety of a specific area maintain treatment records, undertake surveillance of pest populations, and are subject to endangered species habitat impact assessment in treatment zones. Private mosquito management companies often span a wide region and the protected and vulnerable non-target species within this service area can vary. A company that foregoes proper integrated pest management (IPM) practices, will be unprepared to manage endangered species issues. This overview of a private mosquito management operation will highlight the challenges faced by a small-scale operator servicing multiple counties in

Florida and Alabama and present a solution to avoid a potential treatment exposure. The RESPECT Plan is a preparedness and mitigation operations document designed to create awareness and continuity within a service area and take steps to avoid jeopardy to any species of concern. This document is a template that can be scaled to fit all service parameters across multiple districts, companies, regions, or states and assess any species of interest by habitat or migratory route. In the event of a treatment concern, contact lists of key personnel are in place with the response and recovery strategy ready to be implemented. Decisions in times of uncertainty are best applied through protocols and the public relations fallout from poor planning and delayed or inappropriate decisions can be detrimental to pesticide applicators. Endangered species and pollinator protections will continue to increase through public support, regulatory demand, and product label restrictions. An effective planning document adaptable to any pest management scenario is a valuable tool to protect wildlife, the environment, and the integrity of the applicator.

62 **AMCA legislative and regulatory issues and meeting update**

Angela Beehler, angela@mosquitocontrol.org

This presentation will introduce the advocacy topics and goals for the AMCA Washington (D.C.) Conference set to occur in May of 2023. AMCA has made significant progress towards our policy goals over the last year, and the committee looks forward to presenting an update to members. Position statements for the Washington Conference will be decided earlier this year, so you can best prepare for the meeting. This presentation is meant to inform members on how to get involved in AMCA's advocacy efforts and make the most out of your time in D.C.

Special District: Perspectives of Trustees and Commissioners Symposium II

63 **Of Transparency, Contracts and Performance: Navigating the Brown Act, Director Contracts & Performance Evaluations for Mosquito Abatement Districts in California.**

William Donahue

The Brown Act was enacted in 1953 to guarantee the public's right to attend and participate in meetings of local legislative bodies and as a response to growing concerns about local government officials' practice of holding secret meetings that were not in compliance with advance public notice requirements. The Brown Act is pivotal in making public officials accountable for their actions and in allowing the public to participate in the decision making process.

Communication between Managers/Directors and Board Members is critical and starts with a comprehensive contract reflecting employment goals and objectives clearly spelled out and measurable. These expectations should present a clear pathway for what the job entails, accountability to the Board of Trustees and ultimately the citizens of the community for which we serve. Financial compensation should be clearly defined and discussed as well as other benefits associated with employment.

Performance evaluations should reflect agreed upon goals and objectives between the Board and the Director on an annual basis, which are both attainable and measurable. This talk will address experiences encountered from real life accounts of a small California District.

64 **Special District's Challenges for New Managers/Directors – Expect the Unexpected**

J. Wakoli Wekesa

Special district agencies involved in managing and controlling mosquitoes and other vectors are diverse, each with its unique and specific challenges. District Managers/Directors first recruited to oversee these organizations inherits the advantages and all sets of challenges, therein without necessarily ready tools to mitigate them. It requires preparation, patience, and perspectives to bring those challenges under control while maintaining a high-level service to the community. I offer some experiences and observations across several special districts I have been privileged to serve in senior leadership and management roles.

65 **Strategic Planning in District Operations**

Jeremy Wittie, jwittie@cvmvcd.org

Planning is strategic when it helps move an agency from its current situation to its desired future. A three-year Strategic Business Plan (SBP) is the highest-level planning document for an agency and represents the Governing Board's and Staff's direction for the future. The SBP identifies the Agency's mission, vision, and core values while providing a set of goals and objectives that serve as a framework for decision-making. The SBP is also a practical working tool that provides clear direction to the Staff regarding the Board's goals and objectives. It includes a work plan developed by staff to meet those goals and objectives. The Board and Staff regularly refer to it as a guide to the District's actions during the period covered. The presentation will describe how a Southern California Mosquito and Vector Control Agency develops and implements a SBP to achieve its mission.

66 **Doubling the District's size: Two years of outreach, politicking, and patience**

Robin King, rking@cmcd.org

The Collier Mosquito Control District (CMCD) has expanded its boundaries 10 times since 1950 when it was created to serve six square miles. Today's boundaries encompass 401 square miles of a 2,300-square-mile county, and a rapidly growing population is building homes outside those 401 square miles, leaving those residents vulnerable to mosquito-borne disease. When Collier County Commissioners in 2021 approved the creation of new villages outside CMCD's boundaries, the time seemed right to pursue a boundary expansion. According to Florida Statutes, a mosquito control district's expansion only requires approval of the county commissioners in order to advance the local bill to state legislature...but not this time. Two years later -- after approvals, reversals, and a referendum -- CMCD's leadership is hopeful that the expansion may soon be finalized. This presentation describes the outreach, politicking, and patience required during this experience.

67 **Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests**

Elizabeth Andrews, andrews.elizabeth@epa.gov

EPA-registered pesticide products are an important part of pest management programs to accomplish control of invertebrate public health pests. The Agency recently announced a final rule to clarify what efficacy data must be submitted to support registration of certain pesticide products. The

rule, Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests, codifies data requirements to support registration of products claiming efficacy against three categories of invertebrate pests: those of public health importance (e. g., mosquitoes, ticks, cockroaches, and fleas), wood-destroying insects (e. g., termites), and certain invasive invertebrate species (e. g., Asian long-horned beetle). These data document how well the product performs the intended function, such as knockdown or killing, against the invertebrate pest. The content and implications of the new rule for registration of products that target public health pests, such as mosquitoes and ticks, will be discussed.

68 The Grounding of Unmanned Aerial Systems (UAS) in Florida: will the Lee County Mosquito Control District Fly UAS in 2023?

Aaron Lloyd, lloyd@lcmcd.org, David Hoel, Eric Jackson

The Lee County Mosquito Control District (LCMCD) is located in Southwest Florida and was established as an independent taxing district by an act of the Florida State Legislature in 1958. For the last sixty-five years, LCMCD has strived to be at the forefront of mosquito control utilizing a solid base of classic Integrated Mosquito Management (IMM) techniques while developing and/or refining cutting edge techniques sensitive to Florida's unique natural habitat, researching more efficient and effective ways to combat pestiferous and disease vector mosquitoes, and ensuring that Lee County citizens can enjoy a comfortable and safe outdoor environment. To continue the effort in utilizing emerging technologies as potential mosquito control techniques, LCMCD has identified Unmanned Aerial Systems (UAS) technology as having great potential for mosquito control. However, the State of Florida has grounded most operational drones effective January 1, 2023, per § 934.50 (7), Fla. Stat. (2021). This is due to the potential for adversarial countries to hack into unsecured critical UAS components to steal the data. This presentation will discuss LCMCD's legislative and regulatory efforts over the last year to help ensure that UAS will continue to be a tool in the operational toolbox.

69 Barrier treatments methods for residential mosquito management revisited- the backpack fogger is obsolete

Dan Killingsworth, dan.killingsworth@pest.com

Mosquito management of individual properties by private pest control operators primarily entails monthly backpack fogger applications of a pyrethroid residual barrier treatment, often focusing on property line vegetation assumed to be utilized by adult mosquitoes as resting sites. An ideal backpack fogger delivers the product with an optimum mean droplet range of 60-220 microns for deposition on the top and underside of the leaf while ideally minimizing the potential for drift. The velocity of air required to shear the chemical droplet to an adequate size and propel the product to the intended deposition target thrusts a significant percentage of the treatment cloud to unintended sites. As a means of re-evaluating the delivery method for barrier treatments, over 200 residential mosquito service accounts were compared using the Stihl 200 backpack sprayer and the NPD Eliminator E-320 (18 volt) electric backpack sprayer. Assessment of droplet size, target deposition, non-target drift, time per service, volume of product used, cost of product, and re-service requests were undertaken. The NPD Eliminator proved superior or on par with the Stihl 200 fogger in every category. The extended wand and specialized application tip of the NPD system can be placed within the shrubbery canopy and other vegetation at multiple levels and angles. Concerns regarding drift and non-target harm are minimized as

the application tip is near the target area. Calendar based scheduling of mosquito barrier applications, foregoing surveillance efforts, and utilizing a limited arsenal of treatment products with similar modes of action have the potential to increase pesticide resistance and is counter to best management practices. Alternative methods to backpack fogger applications for targeting mosquito populations are necessary to minimize repercussions to pollinators, protected species, and other non-target invertebrates, as well as elevating the standard of professionalism for pest control operators.

Student Competition II

70 Effects of Wolbachia and larval competition on fitness and West Nile virus infection in *Culex quinquefasciatus*

Abdullah Alomar, a.alomar@ufl.edu, Eric Caragata, Barry Alto

Wolbachia bacteria are extremely widespread among arthropods but the roles that native Wolbachia infections play in mosquito biology and vector competence are still to be elucidated. This study evaluated the influence of Wolbachia in fitness and West Nile virus (WNV) infection in *Culex quinquefasciatus* under different levels of larval competition. Mosquitoes experienced longer development time as larval competition increased regardless of the presence or absence of Wolbachia. The presence of Wolbachia promotes adult eclosion under high larval competition stress. High competition leads to loss of Wolbachia density in adults. Although Wolbachia did not affect the susceptibility to WNV infection, it did lower WNV load in adults but only under low larval competition. These findings suggest that native Wolbachia infections can produce fitness benefits by promoting adult eclosion during high stress and reducing viral load when stress is low.

71 Implications of pre-diapause timing on *Culex pipiens* energetic reserve acquisition and overwintering success

Sara Wilson, smw2@illinois.edu

Diapause preparation in *Culex pipiens* is marked by a shift from host-seeking to nectar gluttony to build lipid reserves. An open question is how female mosquitoes resolve the trade-off between the time needed for resource acquisition and the length of the overwintering period. To assess this, a simulated overwintering study was conducted on *Cx. pipiens* females reared under variable temperatures and offered sugar for variable periods. Survival and energetic reserves were assessed.

72 Thermal preferences of *Aedes aegypti* mosquitoes

Olivia Winokur, ocwinokur@ucdavis.edu, Christopher Barker, Claire Chapman

Mosquito-borne pathogen transmission models used to inform control decisions are only applicable if we incorporate the temperatures mosquitoes experience. However, mosquito thermal preferences are not well resolved. We studied *Aedes aegypti* thermal preferences and found that female *Ae. aegypti* generally avoided temperatures >30°C on a gradient in the lab, and chose relatively cooler microhabitats in the field as ambient temperature increased. Incorporating these preferences could improve the accuracy of transmission models for *Ae. aegypti*-borne viruses.

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

Kai Blore, kblore@amcdfi.org, Rebecca Baldwin, Christopher Batich, Philip Koehler,
Roberto Pereira, Cameron Jack, Whitney Qualls, Rudy Xue

Metal nanoparticles have demonstrated strong toxicity against mosquitoes across all life stages and present a promising avenue of research to address the growing development of insecticide resistance in mosquito populations. In this study, nanoparticles were synthesized from silver nitrate using essential oils from different plants. Toxicity screening of these nanoparticles were then conducted via topical applications to assess their viability as standalone insecticides and for potential synergism with existing insecticides.

74 **Host associations of biting midges (Diptera: Ceratopogonidae: Culicoides) at deer farms in Florida, USA.**

Kristin Sloyer, kesloyer@gmail.com, Carolina Acevedo, Samantha Wisely, Nathan Burkett-Cadena

Culicoides spp. are vectors of veterinary pathogens, however their host associations are poorly documented. Bloodmeal analysis was used to determine species-level host associations of 3,603 specimens belonging to 18 Culicoides species at Florida deer farms. We tested for differences in host use between species at farms and compared host use for Culicoides stellifer and Culicoides insignis. Results suggest that while some species exhibit preferences for certain host species, many species are influenced by host availability.

75 **Inhibition of the pentose phosphate pathway to interfere with metabolic mechanisms of insecticide resistance: Evaluation of a potential pyrethroid synergist**

Erin Kelly, etkelly@ucdavis.edu, Geoffrey Attardo

Resistance to pyrethroid based insecticides has facilitated the rapid spread of Aedes aegypti throughout California, and hampered districts ability to suppress West Nile vectors. Resistance in vector insects is mediated by both target site mutations and metabolic mechanisms of resistance. We used high-throughput metabolomics to investigate pyrethroid metabolism in F2 pyrethroid resistant Ae. aegypti from the Central Valley. We then investigate the activity of a novel potential pyrethroid synergist.

327 **Evaluating Metabolic Resistance in Culex quinquefasciatus Across the State of Florida**

TJ Fedirko, fedirko.t@ufl.edu, Eva Buckner, Alden Estep

The major West Nile vector, Culex quinquefasciatus has reportedly high levels of resistance. Bottle Bioassays have shown this to be the case across much of the state of Florida. To understand the mechanisms behind their resistance, an examination of the enzymatic activity in these mosquitoes is vital. The data has shown upregulation in enzymatic families, especially alpha/beta esterase groups. Our results show that Culex quinquefasciatus relies on metabolic resistance, offering broad-spectrum resistance.

Insecticide Resistance - Building a Program Step by Step Symposium I

76 Lessons Learned While Setting Up a Mosquito Resistance Laboratory in a Public Health Department, Phase I

Nina Dacko, nmdacko@tarrantcounty.com

A mosquito's ability to become resistant to adulticides used by mosquito control operations is a major issue, worldwide. Many mosquito control operations perform adulticiding activities, but lack access, personnel, and resources to perform basic testing for resistance to pesticides within their local mosquito populations. This presentation will review a plan to set up mosquito resistance testing in three phases. Phase I, collecting and maintaining wild-type local mosquitoes for testing utilizing CDC bottle bioassays, Phase II, establishing and maintaining a susceptible lab colony, and Phase III, field testing of susceptible and wild-type mosquitoes. Phase I of set-up has been completed. Materials needed for set-up, funding sources, initial personnel training, and location acquisition will be discussed, as will unexpected training hardships including basic colony maintenance, how seasonality of egg collection may affect larval viability, correctly transferring mosquitoes from a breeder to a cage, and other ideas to think about while performing CDC bottle bioassays.

77 Collaboration Combats Insecticide Resistance and Improves Vector Management

Casey Parker Crockett, casey.crockett@azelis.com, Katie Williams, Tre Williams, Alden Estep, Caio Martinelle Franca

Mosquito adulticides are a critical component of mosquito control programs in targeting both vector and nuisance mosquito populations. Monitoring and managing insecticide resistance for mosquito control programs is an important piece of any program as it helps preserve the limited chemical toolbox available for adulticides. However, lab and field data on how products perform against local mosquitoes is not ubiquitously available to all programs. In our collective mission to kill mosquitoes and protect public health, collaboration is a necessity for vector control programs, researchers, and industry members. In June of 2021, caged field trials were conducted in Oklahoma City and were a prime example of this collaboration in action. The main goals were 1) to collect efficacy data to inform management decisions and 2) capacity building for local programs and students. This team was made up of students, professors, public health department employees from Tulsa and Oklahoma City, private industry, and the USDA. This represented the first field cage mosquito control trial conducted in Oklahoma and provided both Health Departments with information related to the efficacy of two mosquito control products against their mosquitoes.

78 Hooah! Standing Up an Insecticide Resistance Testing Program for CONUS Army Installations

Jennifer Carder, jennifer.b.carder.civ@health.mil

The Pesticide Use and Resistance Monitoring (PURM) Branch is housed in the Environmental Health Sciences and Engineering Directorate of the U.S. Army Public Health Center at Aberdeen Proving Ground, Maryland. The mission of the PURM Branch is to advance the readiness and health of Army personnel and communities by assessing and monitoring entomological public health threats resulting from pesticide use; providing consultative expertise; communicating solutions; and

providing support to optimize the Army's Public Health Enterprise. To work toward this mission, the development of a mosquito insecticide resistance (IR) program for Army installations in the Continental United States (CONUS) was revived in 2018 with the Centers for Disease Control and Prevention's Bottle Bioassay as the staple testing mechanism. Marketing and communication efforts have been integral to standing up the program, which, despite logistical complications unique to the military, is actively generating data and is planning to incorporate enzymatic tests in Fiscal Year 2023. The program's challenges and successes, as well as future plans for mosquito IR testing in the Army, will be discussed.

79 **Bioassays and biochemical testing, a comprehensive IR approach**

Constance Darrisaw, darrisaw@lcmcd.org, Kara Tyler-Julian, David Hoel, Aaron Lloyd

Insecticide resistance monitoring is an important part of a comprehensive integrated mosquito management program. At the Lee County Mosquito Control District located in Lee County, Florida, the insecticide resistance program has progressed over the years to include larval bioassays, CDC bottle bioassays, cage trials, and biochemical assays. Larval bioassays are conducted on field-collected larvae of five species of mosquitoes (*Aedes aegypti*, *Aedes albopictus*, *Aedes taeniorhynchus*, *Culex quinquefasciatus* and *Culex nigripalpus*) to monitor the continued efficacy of four different larvicide active ingredients. Bottle bioassays are conducted on adult mosquitoes raised from field-collected larvae of six different species of mosquitoes using four different adulticide active ingredients to monitor for the development of resistance. Field cage trials are conducted following bottle bioassay results to determine the practical resistance level and biochemical assays are conducted to determine resistance mechanisms. A detailed overview of the insecticide resistance monitoring program at the district will be provided.

National perspective into treatment thresholds for mosquito management Symposium I

80 **Review of Action Thresholds from the Literature**

Whitney Qualls, wqualls@amcdf.org, Vindhya Aryaprema

Many different methods are used and new methods are evolving to control nuisance and vector mosquito populations across the world. Implementation of such control methods at the optimal time has been identified as critical to maintaining the populations below unacceptable levels. The establishment of evidenced-based action thresholds is thus encouraged to achieve maximum results under available resources. A review was conducted to identify different action thresholds published worldwide since 2010 and to understand the associated surveillance characteristics. Only 87 publications filtered through the initial database identification of 1,485 publications were included in the review. Thirty publications reported originally generated thresholds, 13 publications reported a clear statistical method to develop an action threshold although there was no hard defined threshold, and 44 publications mentioned only previously generated thresholds that were used in the study. Overall, the thresholds that directly indicate disease transmission conditions outnumbered those that directly indicate mosquito abundance. The majority of the threshold originated in Asia targeting dengue and dengue vectors. The review discusses the possibility of using external data, spatial and temporal

variations in thresholds, the importance of an adequate lead time to initiate control interventions as well as associated surveillance characteristics that would generate better thresholds.

81 The utility of environmental models to help inform entomological thresholds

Lindsay Campbell, lcampbell2@ufl.edu

Determining treatment thresholds for mosquito vectors is an important and ongoing area of research. Although mosquito control districts often have deep institutional knowledge about the patterns and distributions of important mosquito species at a given time period, data driven predictions of abundances often are not available, despite the potential to use routine surveillance trap collections combined with remotely sensed environmental variables to calibrate models. One potential barrier to understanding the potential for model based approaches is a lack of awareness about and training on the utility of model outputs to help inform treatment thresholds. Adding to this challenge is that several modeling approaches exist and inconsistencies in interpretation of output maps can sometimes lead to confusion. Here, we will outline two modeling approaches, providing applied examples, that can be used to provide information about the distribution and abundances of mosquito species under static and dynamic environmental conditions. First, we will outline the utility of species distribution models to predict where habitats may be suitable for mosquito vectors. Second, we will provide an example of a spatiotemporal modeling approach that incorporates time series data. Examples of the different components of these models, including input data, landscape and climate data, model predictions, and interpretation of mapped results will be highlighted.

82 Using entomological and environmental indicators to better understand mosquito disease risk

Mohamed Sallam, mohamed.sallam@usuhs.edu

Environmental and entomological data have been used to understand the species distribution of mosquito disease vectors on a global and local scale. Multiple model approaches were adopted, not only to accurately predict the future distribution of mosquito vectors but also to estimate the exposure risk to disease pathogens transmitted by mosquitoes. The continuous expansion of urbanization and development projects created new suitable habitats for invasive mosquito vectors or assisted in the re-establishment of others in Florida. This prompted the development of new tools to be used in targeted surveillance and control efforts on regional and local scales. However, no standardized surveillance tools have been established to determine the action threshold for control effort. I have compiled from the literature a couple of entomological and environmental indicators to shed the light on the concept and hypothesis of different modeling approaches that affect our understanding of the entomological and environmental thresholds of some mosquito-borne diseases and nuisance caused by filth flies amid natural disasters.

83 Temperature dynamic West Nile virus vector thresholds, St. Tammany Parish, Louisiana.

Kevin Caillouet, caillouet@stpmad.org, Nicholas DeLisi, Josh Foulon, Calvin Smotherman

Surveillance thresholds guide treatment decisions to standardize effort, balance resources, and manage arbovirus risk. Prescribed mosquito control efforts based on data can also limit the spread of insecticide resistance and make more effective treatments. An annual process to review treatment thresholds in 2022 resulted in temperature dynamic thresholds for the management of West Nile virus vectors. Given the impact of temperature on the extrinsic incubation period of WNV and on the development of larval *Culex quinquefasciatus*, we introduced lower abundance thresholds when air temperature increased. The average of the mean daily temperature from the previous 14 days falling into one of five categories < 57°, 57-65°, 66-72°, 73-79°, and >79° F determined whether the historical average vector abundance became the treatment threshold, or the threshold was lowered or raised. Conceptually, treatments outside of peak-WNV season should be limited, and more frequent applications of adulticide would be performed when WNV develops the quickest in mosquitoes. Though treatment outcomes of overall management strategies are difficult to assess, refining our thresholds to account for known variations in virus and vectorial capacity better inform risk assessment and arbovirus mitigation.

84 **Developing And Implementing A West Nile Virus Driven Response Grid System Using Arcgis Hot-Spot Analysis In Orange County, CA**

Kiet Nguyen, knguyen@ocvector.org, Timothy Morgan, Laura Krueger, Sokanary Sun, Xiaoming Wang, Robert Cummings, Amber Semrow

In the wake of Orange County's (California) worst West Nile virus (WNV) epidemic in 2014, Orange County Mosquito and Vector Control District (OCMVCD) took steps to increase sensitivity of early detection of WNV virus activity and decrease control response time. In 2019, OCMVCD developed and implemented a Geospatial Response Identification System (GRIdS) derived from 15 years (2004 – 2018) of spatial data for WNV human cases and virus positive dead birds. Orange County Mosquito and Vector Control District used these spatial data to construct a hot-spot analysis, which identified three Countywide regions based on WNV risk: high, medium, and low. Each area was divided into blocks (one gravid trap per block) with dimensions set to accommodate OCMVCD's ground-based adulticiding resources. Each block was further divided into smaller cells to delineate sections for additional weekly mosquito sampling that was activated upon an initial WNV detection. The area with the highest density of WNV activity, based on human cases and positive dead birds, was assigned a smaller surveillance grid compared to the medium and low risk areas. Mosquito trapping was relocated towards the centroid of each block. Within each risk area, the blocks established a standard unit for calculating infection rates based on routine and extended trapping efforts. During the first implementation year (2019) and subsequent years, the grid system enhanced communication of risk to constituents and stakeholders at a neighborhood level and allowed for a more rapid control response.

Next-Generation Genetic and Biological Approaches to Aedes Control: SIT and Beyond Symposium I

85 **Development of SIT as an IPM tool for the Control of *Aedes aegypti***

Kenneth Linthicum, kenneth.linthicum@usda.gov, Robert Aldridge, Barbara Bayer, Seth Gibson, Rudy Xue, Whitney Qualls, Chao Chen, Daniel Hahn

Aedes aegypti mosquitoes have an increasing geographic range and their significant public health threat as a vector of Zika, dengue, chikungunya and yellow fever viruses is expanding. Control of its immature stages is difficult given the challenge in treating their cryptic development sites. Sterilization of insect pest populations through radiation using Sterile Insect Technique (SIT) has been in use for controlling agricultural pests and has been available for mosquito control since the mid-1950s; however, SIT is not currently used by mosquito districts in the U.S. as a routine control method in Integrated Vector Management plans. We developed methods to sterilize male *Ae. aegypti* mosquitoes with radiation in a SIT program that can be used by mosquito districts in the U.S. as a routine control method in Integrated Vector Management plans. We describe the program and present our findings on the feasibility and impact of deploying an SIT program for the control of *Ae. aegypti*.

86 **Startup costs associated with an X-ray SIT program at Lee County Mosquito Control District**

David Hoel, hoel@lcmcd.org, Rachel Morreale, Aaron Lloyd

Sterile insect technique (SIT) is an older technology that has only within the last several decades gained traction as a control tool useful to integrated vector management. *Aedes albopictus* and *Ae. aegypti* have recently become targets of SIT control programs worldwide. A small scale X-ray sterilization program was begun in 2017 at the Lee County (Florida) Mosquito Control District (LCMCD), after consultation and advisement from the International Atomic Energy Agency, the Centers for Disease Control and Prevention, and the University of Florida. Our program strives for releases of 1,000,000 sterile male *Ae. aegypti* per week. Nine SIT employees are needed to obtain this level of production. This presentation will discuss the startup costs associated with building modifications, capital outlay, equipment & operational expenses, and labor (including employee benefits). From mid-2017 to mid-2020, LCMCD spent approximately \$1.4M before the first releases of *Ae. aegypti* began in June, 2020. LCMCD is a sizeable organization compared to most mosquito control districts within the US, however, our experience in standing up an SIT program demonstrates that with proper budgeting, districts willing to begin with a smaller SIT staff producing fewer sterile mosquitoes could afford to develop an X-ray SIT program with considerably less money.

87 **Progress and challenges for the *Aedes aegypti* (L.) sterile insect technique program on Captiva Island (Lee County, Florida)**

Steven Stenhouse, Stenhouse@lcmcd.org, Rachel Morreale, Johanna Bajonero, Danilo de Oliveira Carvalho, Aaron Lloyd, David Hoel

Lee County Mosquito Control District (LCMCD) founded a sterile insect technique (SIT) program in 2017. After years of entomological baseline collection and laboratory advances to mass rear *Aedes aegypti*, operational releases of sterile males began in June 2020. Suppression of wild *Ae. aegypti* was achieved in a pilot area on Captiva Island through consistent releases of sterile male *Ae. aegypti*. This success led to further expansion of releases to target wild populations of *Ae. aegypti* in new areas further south on the island. While the operational program of *Ae. aegypti* SIT has made progress in the advancement of field activities and releases, there were several factors that complicated these developments. Although these challenges played a significant part in the release capabilities, the SIT department proved to be resilient and recovered well enough to still observe impacts on the population

of *Ae. aegypti* in the expanded release areas. This presentation will discuss the challenges and successes of maintaining an operational SIT program, our current status, and plans for the future

88 Evaluation of X-ray radiation to adult male *Aedes aegypti* – Gainesville, FL strain

Barbara Bayer, barbara.bayer@usda.gov, Robert Aldridge, Jedidiah Kline, Bianca Moreno, Seth Gibson, Chao Chen, Peter Jiang, Daniel Hahn, Kenneth Linthicum

Aedes aegypti is a mosquito known to vector pathogens such as Zika, dengue, chikungunya and yellow fever, which can cause disease in humans and animals. *Ae. aegypti* is also notoriously difficult to control. The use of larvicides and source reduction are good tools to limit *Ae. aegypti* populations. However, these tools are ineffective when trying to control the mosquitoes in the wide range of cryptic breeding locations it utilizes. An additional tool under development to control *Ae. aegypti* has been the Sterile Insect Technique (SIT). In the past, we have used a Cobalt-60 gamma irradiation source to sterilize male pupae. In this study we examine the effect that radiation has upon male adult *Ae. aegypti*, collected in Gainesville Florida, irradiated with X-rays. We discuss the differences we have observed from irradiating adults (Gainesville, FL strain) vs. pupae (AMCD strain collected in St. Augustine, FL), X-rays vs. Cobalt-60, and describe compaction methods of male adults in conjunction with X-ray irradiation.

89 Southern California SIT Joint Pilot Project: Partnering for The Future of Mosquito Control

Steven Vetrone, svetrone@glacvcd.org, Amber Semrow, Tanya Posey, Timothy Morgan, Nicolas Tremblay, Sokanary Sun, Steve Shepherd, Rick Howard, Susanne Klüh

Insect population control using the Sterile Insect Technique (SIT) utilizing x-ray irradiation is not a new method in pest management. This SIT strategy has been successfully used for decades on a variety of pests across the globe. It is, however, a novel approach for use in mosquito control. Projects by groups in Florida, Texas, and Brazil are paving the way for mosquito control agencies to investigate the use of this technology on a local scale. The Orange County Mosquito and Vector Control District (OCMVCD) and the Greater Los Angeles County Vector Control District (GLACVCD) initiated a partnership in late 2021 to conduct an SIT Joint Pilot Project. The projected 5-year pilot project aims to study the effects of releasing irradiated adult male *Aedes aegypti* mosquitoes in urban areas of southern California. This discussion will highlight the steps involved in starting a joint project, outline the study's goals and objectives, and provide an overview of the project's status.

90 Confronting SIT misinformation in Berkeley, California

JUDITH PIERCE, judith@mosquitoes.org, Eric Haas Stapleton, Ryan Clausnitzer, Robert Beatty

The city of Berkeley, CA is a trendsetter for social and environmental movements. In Fall 2021 a Berkeley city councilmember learned about a proposed release of GMO mosquitoes in California from a brief written by an environmental activist organization. In response, the councilmember wrote a resolution for the council to send a letter of opposition to state elected officials and regulatory agencies. While the planned release would target only areas with an established population of *Aedes aegypti*, thereby excluding Berkeley, the city council frequently votes on items which have set precedents for

other cities, and often Berkeley's resolutions attract national media attention. Once informed of the proposed resolution, the Alameda County Mosquito Abatement District assembled a team to meet with the councilmember to address inaccuracies put forward by the activist organization. After conversations with the district staff, the councilmember invited the team to present at the city council meeting. During the council meeting, district staff and board trustees corrected falsehoods from the activist brief, and after an hour-long discussion which included comments from residents and anti-GMO activists, all nine councilmembers voted against the proposal.

The councilmembers realized the letter of opposition was based on inaccuracies and they acknowledged limited expertise on the subject, therefore the vote was a major victory for the district. In the following weeks the district spoke with councilmembers, and the writer of the anti-GMO brief. The issue culminated in an article for *Berkeleyside.com*, a website for local issues, entitled "No, genetically engineered mosquitoes aren't about to be released in Berkeley." This series of events and the outcome demonstrate the importance of maintaining positive relationships with civic leaders. If there are local organized movements against SIT methods, a mosquito district should address the controversy directly, rather than allow non-mosquito organizations to control the narrative with inaccurate information.

91 **Collaborative efforts between Delta Mosquito & Vector Control District and Oxitec in the Central Valley of California**

Mustapha Debboun, mdebboun@deltamvcd.org

The yellow fever mosquito *Aedes aegypti* (Linn.) is an invasive species in California. Since 2013, *Ae. aegypti* has spread and become established in 23 counties in the state, posing unique challenges to mosquito control because of its diel periodicity, proximity to people, and use of cryptic harborages. Delta Mosquito and Vector Control District (MVCD) invited Oxitec Ltd. to evaluate their self-limiting gene technology against invasive *Ae. aegypti* within our District, pending state regulatory approval. This presentation will focus on community education and outreach efforts by Delta MVCD and Oxitec. Thousands of homes were visited, and over twice as many homeowners have signed up to host male mosquito-release boxes and traps to collect adults and larvae. Delta MVCD and Oxitec have attended baseball games, farm markets, fairs, and community clubs to speak directly with homeowners about Oxitec's self-limiting gene technology. The public support for this technology has been widespread and enthusiastic.

Novel Adulticide Space Spray: A Decade of Innovation Symposium I

92 **Introduction and Overview of ReMoa Tri™**

Beth Ranson, beth.ranson@valentbiosciences.com, Chris Byrne

ReMoa Tri™, a triple action mosquito space spray is the result of a decade of innovative research and extensive field testing. ReMoa Tri™ is a breakthrough biorational space spray based on a bacterium, combining three new active ingredients with three different modes of action for effective mosquito resistance management. ReMoa Tri's patented biorational technology assures consistent application and efficacy against both metabolic and *kdr* resistance.

My presentation will be on the three active ingredients and the three modes of action of ReMoa Tri™.

93 **Transient tolerance to pyrethroids in gravid mosquitoes: Implications for viral transmission and ULV control**

Mark Clifton, mclifton@nsmad.com

Mosquito tolerance to pesticides is a complex and multi-layered phenomenon with genetic, metabolic, physiological, behavioral, and even physical mechanisms working together to produce a “resistant” phenotype. Vector *Culex pipiens* mosquitoes in the Chicago, Illinois region routinely demonstrate high levels of tolerance to pyrethroid control materials. Surveys utilizing the CDC bottle bioassay were conducted in 2018, 2019 and 2020 and demonstrated geographically widespread and temporally stable resistance to sumithrin and permethrin. Operational field trials in 2020 with *Culex* spp. mosquito populations and caged field trials in 2021 further corroborated a reduced susceptibility to ground-based ULV methodologies based on pyrethroid materials. Gravid *Culex* spp. mosquitoes, as measured by a BG Counter and modified CDC gravid traps, were especially poorly controlled in field trials. Further wind tunnel trials with resistant and susceptible mosquitoes demonstrated that resistant mosquitoes exhibit an enhanced resistance in the 48 hours after a blood meal that nearly halves their already reduced susceptibility. Metabolic and genetic resistance coupled with a transient post-blood meal resistance is likely to have important implications for the age-structure and viral prevalence of targeted vector mosquito populations.

94 **Evaluation of VBC-60748 against pyrethroid-resistant *Aedes aegypti* and *Culex quinquefasciatus* in Collier County, Florida**

Rebecca Heinig, rheinig@cmcd.org, Rachel Bales, Robert Straser, Atom Rosales, Zachary Nickell, Keira Lucas

Collier Mosquito Control District serves a population of approximately 300,000 residents in southwest Florida. In recent years, Collier and its surrounding counties have experienced outbreaks of not only West Nile virus, which is endemic to the area, but also dengue and Zika viruses. Unfortunately, many local populations of vector species *Culex quinquefasciatus* and *Aedes aegypti* are resistant to pyrethroids, and one *Ae. aegypti* population has also exhibited resistance to naled, which has left the District with limited adulticide options for these vector strains.

The new adulticide VBC-60748 presented a possible alternative. This product combines a fatty acid, the pyrethroid fenpropathrin, and abamectin, a macrocyclic lactone that has a different mode of action than pyrethroids and organophosphates. To evaluate the efficacy of this product against pyrethroid-resistant vectors, the District performed a series of semi-field trials. Local *Cx. quinquefasciatus* and *Ae. aegypti* adults were placed in field cages and challenged with a ground-based ULV application of VBC-60748. Preliminary trial results indicated that VBC-60748 successfully controlled pyrethroid-resistant vector strains and shows promise as an effective option for the District’s integrated mosquito management program.

95 **Data Collection and Considerations in the Drift of a Novel Formulation**

Harold Thistle, hwthistle@gmail.com, Leanne Lake, Banugopan Kesavaraju

Trials have been conducted to evaluate the performance of a novel adulticide including evaluation of drift potential. Measuring drift from the aerial release of fine sprays is an on-going challenge. The primary sampling method included rotary impactors as described at length in this community previously. Fine droplets can move over long distances in the atmosphere so positioning of samplers at distance requires logistical planning and analysis of drift potential using available mathematical models as well as field experience. Avionics assists in the positioning of the aircraft to improve targeting.

The main physical variables influencing drift are generally long known but some questions remain. The possible role of sampler collection efficiency in interpreting data is examined. The role of both environmental variables and specific physical properties of the spray will also be investigated. The combination of experience and modern technology allows insight into insecticide drift not available in until recently but questions remain.

96 **The history of development of ReMoa Tri™; Concept to Reality**

Benjamin Belkind, elizabethranson@gmail.com, Banugopan Kesavaraju

Resistance is a complex issue that necessitates development of novel tools to navigate the physiological barriers developed from repeated selection. Single modes of action that have been around for long periods of time will eventually have less utility. While combining multiple modes of action which are also unique to mosquito control can help enhance public health professional's toolbox, this approach does present a formulation development challenge. Many "small molecule" pesticides (pyrethroids and organophosphates currently available to mosquito control) have molecular weights in the 200 - 500 range. One of the main active ingredients in ReMoa Tri is abamectin whose molecular weight is 3-4 times larger than traditional active ingredients used for space spray formulations. Size exclusion chromatography and nanofiltration are based on molecular weight and effective molecular size. It stands to reason that the same will also influence cuticle uptake, along with other uptake-influencing parameters, e.g. solubility, polarity, HLB, pKa, Log P, etc. The formulations challenge is to integrate all three active ingredients (each with its own mode of action) in ReMoa Tri to coexist in vitro + in vivo, as well as deliver this molecule complex across the cuticle. The presentation will frame the formulation challenges and nuances associated with the development of ReMoa Tri.

97 **Pilot study with ReMoaTM, a new mosquito adulticide, dispersed by thermal fogging against susceptible and pyrethroid resistant Aedes aegypti populations**

Seleena Benjamin, seleena.benjamin@valentbiosciences.com, Guat Ney Teoh, Nazni Wasi Ahmad

Pilot study with ReMoaTM, a new mosquito adulticide, dispersed by thermal fogging against susceptible and pyrethroid resistant Aedes aegypti populations

Seleena Benamina, Teoh Guat Neya and Nazni Wasi Ahmadb

a Valent BioSciences LLC

b Senior Medical Entomologist, Institute for Medical Research, Malaysia

Mosquito adulticide (to be branded as ReMoa™ in Malaysia), a first mosquito adulticide product for Valent BioSciences LLC (VBC), was tested using thermal fogging application against *Aedes aegypti* at the Institute for Medical Research (IMR), Malaysia. The delivery characteristics and the least dosage per hectare that achieves more than 95% mortality was determined for laboratory bred *Ae aegypti* susceptible (S) and pyrethroid resistant (PY) strains. The study was based on the guidelines as provided by WHOPES 1,2. The two test doses were based on the active ingredient Fenprothrin in ReMoa, 1.76 gram and 2.68 gram per hectare, at 1:50 dilution with the diluent provided by VBC. The 2.68 gram per hectare achieved a near 100% mortality in the test populations, including the PY strain, within 1 hour of exposure, for all test distances at 5m, 10m and 15m. The 1.76 gram per hectare had 100% mortality in the S and PY mosquito populations, within 1 hour of exposure at 5m test distance. An 83% mortality was achieved at 24h exposure for the S and PY strains placed at 10m from the spray line. It was also observed that the ReMoa fog cloud suspended in the test site far longer than the 'diesel' based cloud of other adulticides.

1 Space spray application of insecticides for vector and public health pest control. A practitioner's guide. WHO/CDS/WHOPES/GCDPP/2003.5

2 Guidelines for Efficacy Testing of Insecticides for Indoor and Outdoor Ground - Applied Space Spray Applications WHO/HTM/NTD/WHOPES/2009.2

Remembering William (Bill) E. Walton - aquatic ecologist, vector ecologist, leader, colleague, and friend Symposium I

98 Bill Walton – Dedicated leadership and teaching innovation

Alec Gerry, alec.gerry@ucr.edu

Dr. Bill Walton was a remarkably effective and dedicated teacher. He taught several undergraduate classes, though he was particularly fond of teaching Insect Ecology, Introductory Ecology and Evolution, and Aquatic Insects. The student loved these classes, earning Bill top ratings in every course he taught. Bill was specifically recognized for his exceptional teaching prowess through several very prestigious teaching awards. These included being a National Academies Education Fellow in the Life Sciences (2013) and receiving the Outstanding Teaching Award on the UCR campus (2017), from USDA-NIFA (2018,) and from the Entomological Society of America (2018). In addition, the UCR Entomology graduate students awarded him the Faculty Mentor Award in 2010, in recognition of his dedication to mentoring and instruction of our students. Bill's service to entomology and vector ecology is truly remarkable. In particular, Bill served as President of the American Mosquito Control Association and was also a member and Chair of the Editorial Board for the Journal of the American Mosquito Control Association. His service activities are too numerous to include in this abstract but will be reviewed in greater detail during the presentation.

99 Wetlands, climate, and mosquitoes

Dave Heft, dheft@turlockmosquito.com

As a trained aquatic ecologist, Bill Walton spent many years researching natural and man-made wetlands. Bill was particularly interested in the effects of design features and management strategies for constructed treatment wetlands on mosquito populations and water quality performance. Bill also examined how climate, particularly the El Nino – Southern Oscillation, affected mosquito populations in southern California, highlighting the needs for long-term studies to fully appreciate control efforts.

100 **Elemental Stoichiometry of Larval Culex Mosquitoes and the Effect of mosquitofish on the community dynamics of a constructed treatment wetland in Hemet CA**

George Peck, george.peck@dshs.texas.gov

Culex mosquito ecology is complex and incompletely understood. To better understand how biotic and abiotic factors influence larval Culex growth and elemental homeostasis, we conducted two separate but interrelated projects aimed at elucidating the effect of differences in larval food quality and quantity on the growth and whole body stoichiometry of Culex quinquefasciatus and Cx. tarsalis larvae. A second project focused on measuring the effect of larvivorous fish (Gambusia affinis) on the entire aquatic insect assemblage in a constructed treatment wetland. Both projects will be discussed with emphasis on how Bill Walton influenced their design and analysis as well as how the presenter's knowledge of statistical inference and approach to science has evolved. Topics include experimental design, traditional statistical analyses, statistical models and multi-model comparisons, use of R statistical language and R Studio to implement advanced statistical models including full Bayesian model implementation with STAN, use of directed acyclic graphs, and causal analysis to inform scientific discussion and progress.

101 **Following My Passions, a tribute to the teachings and lessons of Dr. Bill Walton**

Adena Why, adena.why@acgov.org

I was privileged to be a graduate student in the lab of Dr. William (Bill) Walton) for over 10 years as I completed both my Masters and PhD. I could not have asked for a better mentor, teacher and researcher to not only learn from but help guide me over all those years. I aspire to continue his work now in my own career in Vector Control. Bill allowed me the freedom to explore my own avenues of research, learn new techniques and develop my projects in my own way. My Masters degree focused on evaluating a fish native to Southern California, the arroyo chub, Gila orcutti, as a potential alternative biological control agent to the Western mosquitofish, Gambusia affinis. The project then morphed into looking at the chemical ecology interactions between the Western encephalitis mosquito, Culex tarsalis, and aquatic predators found in the same habitats. My PhD work marched deeper into the realm of chemical ecology as I worked to tease apart the behavioral responses of Cx. tarsalis to the chemicals exuded from Gambusia affinis. With Bill's guidance and support, I identified two compounds of interest in Gambusia affinis exudate water that the mosquitoes reacted to. I will forever be indebted to Bill for his kindness, tireless patience and warmth through all these years.

102 **A Culture of Collaboration in Public Health Entomology**

Aviva Goldmann, agoldmann@gmail.com

Some of Bill Walton's greatest strengths were in building networks and collaborations across regions and institutions. Throughout his career, Bill built collaborations with sanitary districts that built and managed constructed treatment wetlands; mosquito control districts; wildlife agencies; and academics. Bringing people together to solve public health problems was central to how Bill worked and can be seen in the variety of topics that he actively participated in researching.

103 **Underground Mosquito Control Strategies: Entomopathogenic Fungi and Attractive Toxic Sugar Bait in the Storm Drainage System of the Coachella Valley**

David Popko, david.popko@ucr.edu

The past decade, Bill Walton spearheaded research into two novel, environmentally-friendly mosquito control strategies for underground storm drainage systems (USDS) of the Coachella valley in southern California. The first centered on entomopathogenic fungi applied to USDS walls to kill resting adults and the second on attractive toxic sugar bait (ATSB) stations lethal to sugar-feeding adults. Baseline efficacy tests were established in the laboratory against colony-raised *Culex quinquefasciatus*, the dominate species of wild mosquitoes found at USDS sites. Field studies revealed potential pros and cons of each approach, variable mosquito control outcomes over time and space, and environmental factors that likely shaped the USDS trends. Fungal studies, for example, tracked mortality of *Culex quinquefasciatus* females exposed in overnight chambers to vertical USDS walls treated with a spray formulation of either *Beauveria bassiana*, *Metarhizium anisopliae*, or water. Results over multiple seasons indicated mosquito infection/death varied by fungal species and the age/location/season of each treatment linked to localized and regional fluctuations in temperature and relative humidity. Secondly, ATSB studies with CDC-UV light traps (no carbon dioxide) indicated numbers of wild adult mosquitoes with bait dye varied by station design, age, and USDS structure. ATSB treated with *Beauveria bassiana* resulted in a spread of fungal infections in adults collected within and between USDS chambers, and residual infection after an overwintering period. Both control techniques are predicted to be enhanced in deeper USDS substructures protected from weather and human activity. Such sites would offer more stable temperatures and relative humidity and adult mosquitoes would be expected to take refuge in these conditions to enhance interception rate with treatments. Overall, surprisingly, experimental treatments were not associated with relative declines in wild mosquito production in weekly sampling regimes. The potential viability of fungus- and/or bait-based adulticides in mosquito abatement programs for USDS will be discussed.

Insecticide Resistance - Building a Program Step by Step Symposium II

104 **A Comparison of the Topical Application Assay and the CDC Bottle Bioassay Kit in *Culex quinquefasciatus* Mosquitoes from New Orleans, Louisiana.**

Erin Cloherty Duvernay, ercloherty@nola.gov, James Ottea, Kristen Healy, Claudia Riegel, Janet McAllister, Mark Janowiecki

Monitoring the development of insecticide resistance and identifying mechanisms and risk factors that confer resistance in a vector mosquito population are crucial to an integrated mosquito management program. The first step of an insecticide resistance monitoring program is to screen for insecticide susceptibility. There are various screening methods available.

The objective of this study was to screen local adult *Culex quinquefasciatus* mosquitoes throughout Orleans Parish, Louisiana for insecticide resistance by comparing a topical application assay and the Centers for Disease Control and Prevention's (CDC) bottle bioassay kit. In 2021, wild-type *Cx. quinquefasciatus* egg rafts were obtained from 13 sites within Orleans Parish, LA. Adult mosquitoes that emerged from those egg rafts were screened for susceptibility to bifenthrin, deltamethrin, malathion, and naled, using technical grade active ingredients.

Results from these two assay methods were compared to assess the association between susceptibility outcomes. For topical assays, the mean mortality at 24 h of adult wild-type *Cx. quinquefasciatus* mosquito populations at all 13 sites was 28% for bifenthrin, 26% for deltamethrin, 35% for malathion, and 87% for naled. For the CDC bottle bioassays the mean mortality at the diagnostic time of adult wild-type *Cx. quinquefasciatus* mosquito populations at all 13 sites was 16% for bifenthrin, 7% for deltamethrin, 63% for malathion, and 95% for naled. In conclusion, both assays can detect insecticide resistance, however the results are not uniformly correlated.

105 **Statewide testing of *Culex quinquefasciatus* from Florida**

Eva Buckner, eva.buckner@ufl.edu

From 2019 to 2022, *Culex* mosquito egg raft collection kits were shipped to any interested programs in Florida. The egg rafts received were reared to adulthood and tested for insecticide resistance to the following adulticide active ingredients using the CDC bottle bioassay: permethrin, deltamethrin, etofenprox, sumithrin, malathion, and naled. Using the CDC's guidelines, in general, most *Culex quinquefasciatus* from Florida were resistant to the permethrin, deltamethrin, etofenprox, sumithrin, malathion, and naled. While most populations were found to be resistant, their levels of resistance varied. Naled and malathion usually both resulted in 100% mortality by the end of the assays. Permethrin was usually the best performing pyrethroid active ingredient. Resistance management strategies specific to each population tested based on the results were provided to control programs. These resistance management strategies included rotating active ingredients, increasing application rates, as well as using source reduction and larviciding to decrease the amount of adulticides needed.

106 **The Big Picture – impact of IR testing on WNV Control decisions**

Justin Harbison, jharbison@luc.edu, Mark Clifton

Recently, investigations into the effectiveness of truck-mounted ULV sprays in the Chicago area suggest that even at full label rate these sprays may yield less than ideal results in local *Culex* mosquitoes. Additionally, rebounds in *Culex* abundance above pre-spray levels after a temporary decrease have been consistently observed with these sprays. By leveraging resources from the CDC, subsequent IR bottle bioassays have helped to explain why achieving optimum results with these sprays are challenging. Bioassays performed in and around the Chicago area have confirmed resistance to nearly every active ingredient available, including pyrethrum, deltamethrin, permethrin, sumithrin, and malathion. With this information in hand, the North Shore Mosquito Abatement District has transitioned away from routine ULV sprays to better conserve this tool as a public health response and, concurrently, has added other tools to target larval stages of nuisance and vector mosquitoes. The bottle bioassays will continue to be a useful resource for local programs in the Chicago area.

National perspective into treatment thresholds for mosquito management Symposium II

108 Development Of Wnv Area-Wide Control Triggers For Grided Surveillance System In Orange County, CA

Timothy Morgan, tmorgan@ocvector.org, Kiet Nguyen, Laura Krueger, Sokanary Sun, Xiaoming Wang, Robert Cummings, Amber Semrow

The Orange County Mosquito and Vector Control District (OCMVCD) modified its long-standing West Nile virus (WNV) surveillance program in 2019 with the aim of evolving a more equitable, transparent, and evidence-based disease risk response program. Using spatial analysis tools, we examined the distribution of historical WNV positive humans and dead birds to delineate three risk areas in Orange County, CA, designated high, medium, and low. In each risk area, blocks were formed from nine grid cells and a routine trap site was established in the center, resulting in an equidistant trap distribution. Upon detection of WNV, additional traps were deployed around the centralized site, for a fine-scale evaluation. This discussion details the development of WNV control triggers used to manage area-wide truck-mounted ultra-low volume (ULV) space spray applications within the grided system. The triggers were developed using a geospatial analysis of historical WNV human cases and associated vector indices (VI) on a relatively fine spatial scale. VIs from 2004 to 2018 were cumulated by disease week for outbreak and non-outbreak years separately and compared graphically. The lowest VI threshold that was associated with elevated human cases in outbreak years (≥ 40 cases/yr) was selected as a treatment trigger, a VI of 800. A brief overview of results from a controlled urban treatment study, while operating under the new program, is presented. In conclusion, OCMVCD's surveillance grid and evidence-based trigger response plan has worked to detect early season hotspots and successfully reduced WNV mosquito infection rates.

109 Development of Adult Control Activation Thresholds at the Toledo Area Sanitary District

Jennifer Shimola, jshimola@toledomosquito.org

Adult mosquito control is the most visible activity conducted by the Toledo Area Sanitary District (TASD). As such, the development of science-based activation thresholds for adult control applications is critical. TASD uses historical surveillance data to increase the objectivity of thresholds. Data is collected at fixed locations for four to seven days per week via New Jersey light traps and gravid traps. Therefore, activation thresholds can be location specific and/or temporally specific. Calculation of activation thresholds differs for nuisance and disease carrying mosquitoes. Nuisance mosquito thresholds solely emphasize mosquito abundance in New Jersey light traps and are calculated with averages. Disease carrying mosquito thresholds are more complicated as vector abundance is available quickly, but is not directly related to viral presence. Statistical models assessing West Nile virus presence and vector abundance are used to establish gravid trap thresholds. Furthermore, these thresholds are variable within a season as the relationship of virus and vector changed over time.

110 Implementation of Activation Thresholds at the Toledo Area Sanitary District

Jacob Sublett, jsublett@toledomosquito.org

Activation thresholds can be used as guidelines for mosquito management, indicating when and where mosquito control applications are warranted. Once activation thresholds have been developed and are established within an organization, surveillance data needs to be made

available to staff members in ways that are expedient and easy to interpret, especially in relation to reaching or exceeding thresholds. Moreover, the documentation of thresholds used to initiate treatments are important to maintain within record-keeping as it may give insight into ongoing or future mosquito control issues. The implementation of control in response to an activated threshold depends on a wide variety of factors such as environmental conditions, previous control activities, operational feasibility, the demand or need for treatment in other parts of the service area, and technology capabilities. The practical application of activation thresholds at the Toledo Area Sanitary

District, a mosquito control agency located in Northwest Ohio, will be discussed.

111 Setting action thresholds for larvicide and adulticide applications throughout the Florida Keys

Andrea Leal, aleal@keysmosquito.org, Mikki Coss

Action thresholds are essential to many mosquito control operations throughout the world. The Florida Keys Mosquito Control District utilizes action thresholds prior to any operational ground or aerial application of larvicides and adulticides. Multiple factors are taken into consideration when setting action thresholds throughout the Florida Keys, starting with Florida State law. Florida State Statute dictates when and where a control program can make applications based on multiple methods of surveillance. Other considerations when setting action thresholds in the Florida Keys includes rainfall, presence/absence of mosquitoes, mosquito-borne disease threats, and coordination with both State and Federal land managers. All thresholds are specific to different areas of Keys and can vary by island or neighborhood due to differences in potential mosquito-borne disease threat, base population of mosquitoes, and endangered species concerns. Action thresholds play an important role in operations for the control of mosquitoes, especially throughout the Florida Keys due to all of these complicating factors.

112 Ready, Set, Action Thresholds in Williamson County, Texas

Jason Fritz, jason.fritz@wilco.org

Action thresholds for adult mosquito control in response to detection of West Nile virus (WNV), are created specifically for each jurisdiction. Factors that influence these thresholds include public health risk and public acceptance to adult mosquito control methods. Historically, the Integrated Vector Management (IVM) Program at Williamson County and Cities Health District (WCCHD) has managed responses to WNV detection on a case-by-case basis with treatment decisions determined by affected municipalities. As more guidance and data have become available, the IVM Program has created data-driven action thresholds that can be shared with partner cities to guide in decision making related to adult control. Mosquito and human WNV data from 2018-2021 were analyzed to create and evaluate action thresholds for adult mosquito control in response to WNV detection. Trap abundance, trap positivity, and infection rates were calculated during each WNV detection in mosquito samples and compared at a “regional” versus county-wide level. A table was created to supplement WCCHD’s Phased Response Guidelines for WNV with action thresholds and control recommendations based on the risk

presented at the time of WNV detection. Action thresholds will be evaluated annually and adjusted as needed.

Next-Generation Genetic and Biological Approaches to Aedes Control: SIT and Beyond Symposium II

113 Reinfestation of *Aedes aegypti* following the Debug Fresno program

Jodi Holeman, jholeman@mosquitobuzz.net

In 2018 the Consolidated Mosquito Abatement District (CMAD) along with its collaborators Verily and MosquitoMate demonstrated the successful suppression of female *Aedes aegypti* mosquitoes in an urban residential neighborhood in Fresno County, California through the continuous release of *Wolbachia* infected male *Ae. aegypti* mosquitoes. In 2018 the Debug Fresno program achieved >95% suppression across all release sites with the most isolated site named, Harlan Ranch achieving 98% suppression of wild *Ae. aegypti* mosquitoes. Releases in Harlan Ranch were conducted in 2018 and 2019. The experimental use permit the District and collaborators had been conducting release under expired at the end of 2019. With no additional releases planned, the District established fixed surveillance sites using BG sentinel traps in 2020 to monitor how quickly the population of *Ae. aegypti* recovered in this neighborhood. The outcomes of that surveillance effort will be reviewed in addition to resident feedback the District has received on the Debug Fresno program since 2020.

114 Wolbachia infected males for suppressing populations of *Aedes aegypti* and *Aedes albopictus*

Stephen Dobson, sdobson@mosquitomate.com

For more than a decade, MosquitoMate has worked with mosquito abatement districts to develop and test novel autocidal control technologies against invasive *Aedes* mosquitoes, including field trials in multiple states that target *Ae. albopictus* and *Ae. aegypti*. While federal Environmental Protection Agency (EPA) approval was granted for the MosquitoMate *Ae. albopictus* tool in 2017, the federal permit was time limited to five years and will expire at the end of 2022. In April of 2020, MosquitoMate submitted data to the EPA requesting an extension of the permit for both additional time and an ability to operate in additional states. Concurrently, MosquitoMate submitted an application for *Wolbachia* infected *Ae. aegypti* as a mosquito suppression tool. An application to the California Department of Pesticide Regulation (CDPR) was also submitted for the *Ae. aegypti* tool. At the time of submitting this abstract, both applications remain under review at the EPA and CDPR. This lecture will provide an update on recent regulatory events and the status of new tools that facilitate autocidal technologies, including both *Wolbachia*- and non-*Wolbachia* control approaches.

115 Infection by wMel *Wolbachia* alters female post-mating behaviors and physiology in the dengue vector mosquito *Aedes aegypti*

Catalina Alfonso-Parra, catalfonso@gmail.com, Jessica Osorio, Sara Villa-Arias, Carolina camargo, Luisa M Barrientos, Luis Felipe Ramírez-Sánchez, Stephen Dorus, Frank W. Avila

The globally invasive mosquito *Aedes aegypti* disseminates numerous arboviruses that impact human health. A promising method to control *Ae. aegypti* is transinfection with the intracellular bacterium *Wolbachia*, a symbiont that naturally infects ~60% of insects but is normally absent from *Ae. aegypti*. Transinfection with wMel *Wolbachia* induces cytoplasmic incompatibility, allowing infected individuals to rapidly invade native populations. Further, *Wolbachia* infected females are suppressed for their ability to transmit arboviruses. Thus, wMel infected *Ae. aegypti* are being released in several areas to replace native populations to suppress diseases spread by this species. *Wolbachia* is reported to have minimal effects on *Ae. aegypti* fertility, but its influence on other processes related to reproduction is unknown. Female insects undergo several physiological and behavioral changes in response to mating that are required for optimal fertility referred to as female post-mating responses. Female post-mating responses can be modified by environmental factors, such as nutritional status and microbiome composition. To assess how *Wolbachia* infection influences female *Ae. aegypti* post-mating responses, we collected *Wolbachia*-infected *Ae. aegypti* in Medellín, Colombia, and backcrossed this strain for 7 generations to our laboratory strain. We found that *Wolbachia* influences female fecundity, fertility, longevity, and re-mating incidence, with some effects observed in a sex-specific manner. Changes in female post-mating responses are not due to defects in sperm transfer by infected males, or sperm storage by infected females. Thus, artificial infection by wMel *Wolbachia* influence post-mating processes in *Ae. aegypti*, potentially influencing control programs that utilize *Wolbachia*-infected individuals.

116 **The design of an industrial production line aiming for cost reduction of sterile mosquitos on large scale operations**

Hanan Lepek, Hanan@senecio-robotics.com

Sterile Insect Technique remained a high-cost solution and not affordable on large scale for majority of mosquito abatement districts, while being very effective as the mosquitoes cannot develop resistance against sterility, such as witnessed with insecticides.

With the introduction of automation, advances in the design of Senecio Robotics factory for the packaging of male mosquitoes will be discussed and how automation, robotics and artificial intelligence are incorporated into a single comprehensive solution lowering the cost per single mosquito by orders of magnitude.

The factory suggests optimizing how mosquitoes are being reared today, towards a leaner process, with higher yields and lower number of employees required. During the lecture, the proposed process will be discussed along with presenting the latest technical results.

Join the presentation to educate yourself on sterile mosquitoes and its potential availability as a low-cost tool in the near future.

117 **DO. OR DO NOT. THERE IS NO "TRY" (YODA).**

Nitzan Paldi, nitzan@forrestinnovations.com

THE DATA PROVING THE EFFECTIVENESS OF SIT DEMONSTRATES UNEQUIVOCALLY THAT IT PREVENTS DISEASE TRANSMISSION VIA EFFECTIVE SUPPRESSION OF MOSQUITO POPULATIONS. SIT COMES IN DIFFERENT 'FLAVORS' AND 'COLORS', SOME ARE PROBABLY BETTER THAN OTHERS, BUT OVERALL COMMUNITIES JUST NEED TO IMPLEMENT WHATEVER IS THEIR FAVORITE SIT METHOD. EXISTING Stakeholders SHOULD UNITE AND CONSOLIDATE IN ORDER TO Make SIT THE LEADING TOOL IN MOSQUITO-BORNE DISEASE PREVENTION

118 **Practical field application of Oxitec self-limiting gene technology against *Aedes aegypti***

Rajeev Vaidyanathan, rajeev.vaidyanathan@oxitec.com

The mosquito *Aedes aegypti* is an invasive species in North and South America. This mosquito is an important global vector of the viruses that cause dengue, yellow fever, Zika, and chikungunya. This species feeds almost exclusively on humans and feeds and oviposits close to human habitations, especially in cryptic natural and artificial containers. Throughout its range, *Aedes aegypti* populations are resistant to pyrethroids, the class of insecticides most commonly used to control adult mosquitoes. For these reasons, *Aedes aegypti* is a difficult mosquito to control by traditional larviciding, adulticiding, and larval site disruption. We will discuss former and ongoing projects that assess a self-limiting gene approach to control this invasive species in Brazil and the Florida Keys. Our projects have assessed the flight radius of our male mosquitoes, their mating success with local female *Ae. aegypti*, oviposition behavior of mated females, and the persistence of the transgene during and following male release.

Novel Adulticide Space Spray: A Decade of Innovation Symposium II

119 **Efficacy of ReMoa Tri against resistant malaria vectors in Africa, *Anopheles gambiae* ss and *An. arabiensis***

Dismas Kamande, dkamande@ihi.or.tz, Silas Majambere, Sarah Moore, Jason Moore, Banugopan Kesavaraju

The objective of this study was to compare the bio efficacy of a new mosquito adulticide ReMoa Tri applied by ULV spray against pyrethroid susceptible and resistant *An. gambiae* ss and *An. arabiensis*, two of the main vectors of malaria in Africa.

Fully pyrethroid susceptible *Anopheles gambiae* s.s. (Ifakara), knockdown resistant *An. gambiae* (Kisumu) and metabolic resistant *An. arabiensis* (Kingani) were used in this study. The mechanism of resistance is both 1014F kdr West and 1014S kdr East for *An. gambiae* (Kisumu KDR) and upregulation of CYP450 for *An. arabiensis* (Kingani).

The semi-field bioassays were performed at the Vector Control Product Testing Unit (VCPTU) testing facility located at the Bagamoyo branch of Ifakara Health Institute (IHI), Tanzania (6.446°S and 38.901°E).

Following WHO guidelines, cages were set at 25, 50, 75 and 100m in a block randomized design and three replicates were conducted per distance for each mosquito strain. The ULV backpack sprayer fontan® Portastar S was used to apply a droplet size of (Dv0.5) 12 to 24-micron volume median diameter

(VMD). The trial was conducted at dusk when the wind speed was 3-10 kilometers per hour (kph) with 5kph optimal wind speed. The ULV machine was walked across the test plot at 3.5 kph. All 3 replicates of the spraying were conducted on the same night.

After exposure, outcomes of knockdown at 60 minutes, mortality at 24 and 48 hours were recorded. The mosquitoes were held under controlled conditions at 27 ± 2 °C temperature and relative humidity of $80 \pm 10\%$. A test was valid when control mortality was less than 20% at 24-hours.

Results show 99-100% mortality across the board – in both susceptible and resistant strains of both species. These results and their implications will be discussed.

120 **ADrop™ – Droplet analysis tool for analyzing fluorescent adulticide droplets using open source software**

Banugopan Kesavaraju, banugopan.kesavaraju@valentbiosciences.com, Katie Williams, Leanne Lake

Adulticide operations rely on small droplets impinging on a flying mosquito. The intended target for the spray is the air column just above terrestrial habitats where the mosquitoes will be flying during their most active time. Measuring droplet size of the adulticide spray is an important necessity to make sure that the spray reaches the intended target. Droplet sizes can be gathered from sprayers as soon as they leave nozzle tip with a machine like KLD labs DC IV unit. Reports from this unit can be used to satisfy the calibration requirements for a handheld or truck mounted machine. But the DC IV units can seldom be used for characterizing aircraft especially if they have specialized atomizers that require the aircraft to be flying. Droplet impingers can be used for aerial equipment characterization. ADrop is a free software tool that is available to size and count droplets from slides or rods. Instructions and suggestions to set up a droplet analysis lab will be presented.

121 **Understanding Metabolic and Knockdown Resistance and Their Impact**

Chris Byrne, christopher.byrne@valentbiosciences.com, Beth Ranson

In biological terms, resistance can be defined as the natural ability of an organism to withstand a damaging agent or adverse condition. Animals, plants, and microbes have all demonstrated the ability to develop resistance, with either positive or negative outcomes, depending on the interaction. In terms of mosquito insecticide resistance, it can be defined as the ability of a mosquito to survive exposure to a standard dose of insecticide.

Mosquitoes that are heavily selected with pesticides tend to evolve two major types of resistance: metabolic resistance and knockdown resistance. Mosquito resistance means decreased efficacy and an increased threat of vector-borne disease.

122 **“Conducting Aerial Adulticide Efficacy Trials”**

Leanne Lake, Leanne.Lake@valentbiosciences.com

The need for the development of new active ingredients for the control of adult mosquitoes is an ever-growing concern for those that deal with pyrethroid resistance, which poses one of the more serious threats to integrated pest management programs throughout the world. Valent BioSciences Mosquito Adulticide project has been in the works for many years and the development of

this new product represents the first novel adulticide mode of action in the US in nearly 50 years. An Experimental Use Permit (EUP) was approved by the US EPA for aerial applications of the new adulticide targeting pyrethroid-resistant mosquitoes. Evaluations of the three active ingredients have been ongoing and currently we are investigating the efficacy of aerial applications with caged mosquito field trials according to the EPA requirements. The test design layout, data collection, droplet analysis methods, and flight offsets used for successful evaluations will be covered. Additionally, a review of equipment set-up, calibration methods and spray parameters used for every trial conducted in 2022 will be presented.

123 **ReMoaTri Technical Tips**

Katie Williams, katie.williams@valentbiosciences.com, Leanne Lake, Banugopan Kesavaraju

ReMoa Tri, a novel biorational combination with fermentation based avermectin, a Type II pyrethroid and fatty acids, provides broad spectrum efficacy for integrated pest management programs and helps to manage insecticide resistance. ReMoa Tri can be applied by ground and aerial Ultra-Low volume sprayers. This new adulticide is easy to use and can provide a positive operational experience. ReMoa Tri is the first fermentation based adulticide space spray and has three active ingredients. Because the formulation is so unique, this presentation will provide some technical tips to follow when conducting operational applications.

Remembering William (Bill) E. Walton - aquatic ecologist, vector ecologist, leader, colleague, and friend Symposium II

124 **The Maestro of Midgeville: how one man and one plot brought field research to the doorstep of generations of students**

Benjamin Nyman, bnyma001@ucr.edu

Nestled in the heart of the sprawling Agricultural Operations of UCR is a peculiar patch of land. Not a citrus grove or turf grass plot, this area is surrounded by towering trees that create a certain magical seclusion. Known affectionately as “Midgeville” after its original Chironomidae use in the 1970s, a Dr. William Walton joined UCR some years later and began a prolific research program that incorporated this peculiar space for a variety of projects. Following his work, various sizes of experimental ponds, mesocosms, tubs, and tanks fill the clearing today. The flexibility of the space allowed for a study scale more controlled than the field sites so often also utilized. Yet they were large enough to capture a broader range of interactions that mimicked nature much more than bottles and tanks. Midgeville allowed Bill Walton to tease out the ecological interactions that so intrigued him and his students in a variety of ways you will hear about in this symposium, and as his program grew, so did the uses he found. As his research turned to chemical and biological control of mosquitoes, the ponds and tubs became invaluable experimental testing units. Expanding the critical step in understanding the effects of control measures between lab trials and field deployment for the safe mitigation of mosquito-borne disease. From entire simulated wetlands to single leaf axils, Bill fostered this amazing space for

decades of research on aquatic habitats. Without him, it stands as a testament to his work, and hopefully a conduit for the next generation.

125 **Science Communication: Bill as a Leader in Teaching**

Jennifer Henke, jhenke@cvmosquito.org

Bill Walton was an exceptionally effective and dedicated educator. He received multiple awards (National Academies Education Fellow in the Life Sciences; Outstanding Teaching Award at UCR; USDA-NIFA; and the Entomological Society of America). Bill valued students from biology majors in their introduction to ecology, to upperclassmen in insect ecology and aquatic insects. He routinely explored new methods and tools to ensure that students understood and applied concepts. Routinely, students who attended Bill's office hours would find themselves touring his lab so that they learned more about the relationship from first principles to applied research. Related to this work, Bill was exceptional at explaining the importance of the research to non-scientists, ensuring that his audience value the impacts of local research in their communities.

126 **Leaving a legacy: creating opportunities for the next generation of vector ecologists**

Christopher Barker, cmbarker@ucdavis.edu

Dr. William Walton was a founding co-director of the Pacific Southwest Center of Excellence in Vector-Borne Diseases, known as PacVec. Dr. Walton was a critical driving force in the formation of PacVec, and the center is a natural extension of his long history of research, training, and service alongside colleagues in mosquito control. Throughout his career, Dr. Walton was dedicated to inspiring and training the next generation of public-health entomologists. His professionalism and ability to work well with a wide range of people made him an effective leader in AMCA, PacVec, and his own department at the University of California, Riverside. It is a testament to his abilities as a mentor that many of his past trainees still work in mosquito control or public health today. This presentation will consider Dr. Walton's role in PacVec and other efforts that continue to create exciting opportunities for early-career professionals in mosquito control and public health-related research.

Overview of Applied Research Programs at the District/Program Level Symposium I

127 **Assessing past and present applied research projects conducted in Manatee County, Florida**

Samantha Ramirez, s.ramirez@manateemosquito.com, Mark Latham, Jacob Hart, Christopher Lesser

Efficacious and environmentally sound insecticide applications are a necessity to all mosquito control agencies. Early mosquito control research has come a long way from using equipment not suited for versatile mosquitoes and different habitat types. At Manatee County Mosquito Control District (MCMCD) research is year-round with various projects involving the control of a range of mosquitoes such as *Aedes aegypti*, *Ae. taeniorhynchus*, and *Culex nigripalpus*. Nearly 25 years after MCMCD's first research projects analyzing high pressure systems and spray cloud movement, the

evolution of insecticide resistance and new technologies has signified the importance of not only precise field applications but resistance monitoring. While most procedures and equipment have stayed constant throughout the years, some have been altered due to chemical incompatibility of newer formulations, supply-chain shortages, or simply because they were made obsolete. This is an overview of our current research program and major projects over the last five years with an emphasis on efficient procedures, preparation of staff and budgets for various study setups, and technical adjustments made along the way.

129 Field trials and resistance testing in Maricopa County

James Will, James.Will@maricopa.gov, John Townsend

Maricopa County is in the south-central part of Arizona and is the fourth most populated county in the United States. It contains about 62% of Arizona's population, making Arizona one of the most centralized states in the United States. Encompassing 9,300 sq miles, it is the 15th largest county in the nation and one of the fastest growing over the last 10 years. New construction and habitats created for aesthetic reasons have brought additional challenges to our program. On top of that, the monsoon season and flood irrigation bring other concerns. We larvicide 1000's of routine sites monthly and treat between 2k-10k green pools every year. With the number of treatments made annually in an area of this size, we are always concerned with resistance issues. Over the past several years we have been performing field trials and larval resistance testing on our local mosquito populations. We will discuss the findings and results in this presentation.

130 What exactly do you do during the winter months as a northern MAD?

Patrick Irwin, pirwin@nwmadil.com

I am constantly asked what I do during the winter months when there are no mosquitoes out. Two simple words are my response - Collaborate and Plan! The Northwest Mosquito Abatement District is lucky to have amazing collaborators in our area, state, Midwest region and nationally. Many of these projects are spurred on by being a partner with the Midwest Center of Excellence for Vector-Borne Diseases (MCE-VBD). Our partnership with MCE-VBD allowed us to host a graduate student to work on several important research questions. We get to leverage our surveillance and operations to provide data and samples to our collaborators. In return, we get access to world class professors, researchers, graduate, and undergraduate students interested in all aspects of vector-borne diseases. Our collaborators have skill sets, laboratory facilities and resources to help us answer questions we cannot answer by ourselves. I'll be discussing many of these collaborations and results.

Resist the urge to panic: Managing resistance in field operations Symposium I

131 The Integrated Vector Management approach to effective resistance management

Alden Estep, alden.estep@usda.gov, Neil Sanscrainte

Integrated vector management (IVM) uses multiple methods to target multiple life stages for effective control of public health pests over an extended period. Good IVM practice is the most effective means for long-term control of vectors and, when effectively done, can reduce the risk of

vectored disease. However, insecticide resistance is increasingly common and insecticide resistance can reduce the efficacy of both larval and adult chemical control, the two primary methods available for rapid reduction in vector numbers. Understanding the implications of insecticide resistance on the efficacy of IVM is more difficult but we clearly must become adept at managing vectors in an environment with significant resistance.

In this presentation, we briefly discuss how insecticide resistance reduces the efficacy of operational interventions, how resistance surveillance becomes an even more important part of an effective IVM program, and how to reduce the impacts of insecticide resistance on the overall efficacy of the IVM strategy.

132 Outbreak! When you're under a microscope without insecticide resistance data

Janet McAllister, jvm6@cdc.gov, Cassie Scott, Roxanne Connelly

Knowing if insecticide resistance is present in an area before an outbreak occurs is critical. During routine years mosquito control usually does not garner much public attention. During an outbreak, not only do routine control activities intensify, but there is often increased scrutiny by public officials, the press, and the public. This was true in Miami, FL during the 2016 Zika virus outbreak. The Miami Dade Mosquito Control program was conducting intensive mosquito control against *Aedes aegypti* under the assumption that because they never sprayed where this mosquito was causing disease, there was no insecticide resistance. Cases continued despite their intensive control efforts in several concentrated control areas and with the added stress of dealing with outside scrutiny. Insecticide resistance testing was initiated to determine the most effective chemicals and application rates needed to kill *Ae. aegypti* in the area. Once insecticide resistance was detected in both lab and field trials, effective spray strategies were developed to better control local mosquitoes. Resistance was found to varying degrees for all pyrethroids tested but mosquitoes were susceptible to organophosphates. The strategy adopted included house inspections and treatment as needed, aerial spraying with naled, truck spraying with deltamethrin (at an effective application rate) and area-wide larviciding (WALS) using both trucks and aircraft.

133 Mechanism matters: Efficacy of commercial formulations and the relationship to underlying insecticide resistance mechanisms

Neil Sanscrainte, neil.sanscrainte@usda.gov, Alden Estep

Insecticide resistance (IR) in mosquitoes continues to increase, making effective control of the most important human vectors difficult throughout the Southern US. Varied resistance intensity is often found within districts and multiple biological mechanisms are responsible for resistance to different pesticide classes. In *Aedes aegypti*, the knock down resistance (kdr) gene mutations have been found to be indicative of pyrethroid resistance in populations from Florida, Texas, Arizona, California, Mexico and Peru. When kdr is the primary underlying IR mechanism, testing dead or live samples by allele-specific PCR provides a cheap, fast, and highly predictive method to determine a susceptibility to pyrethroids. These data, when compared to kdr genotype specific field efficacy studies, will then determine if the pyrethroid formulation will be effective, or if switching to another active ingredient, such as an organophosphate, is necessary to produce high mortality. In other species, like *Culex quinquefasciatus*, where enzymatic mechanisms are the major IR factor, wind tunnel tests using local

mosquitos can provide data on the efficacy of field sprays of formulated product. If adulticide testing proves to be ineffective due to a local mosquito population's IR mechanism(s), efforts can then be put into controlling other life stages, i.e. treating larval habitats with microbial larvicides or insect growth regulators. As IR is most pronounced on efforts to control adult mosquitoes, determining which formulations are most effective for local populations is crucial to avoid further intensification of IR and the distribution of ineffective chemicals to the environment. This can only be accomplished if IR, and the mechanisms involved, are assessed regularly, and then these data are used to inform control efforts.

134 **Linking resistance monitoring data to control: a framework for addressing critical knowledge gaps**

Laura Harrington, lch27@cornell.edu, Lindsay Baxter

Resistance monitoring is an important component of vector management; however, the link between detected resistance levels and operational control failures is unclear. This link is critically important for sustaining the efficacy of mosquito control practices in the United States and globally, yet it is difficult to address. In addition, the lack of active ingredients available for operational mosquito control underscores the urgency of developing strategies for resistance management within integrated mosquito control programs. In this presentation, we highlight key questions that must be addressed in order to understand linkages between resistance status and control outcomes. We will present ideas on frequency and spatial scales for monitoring risk. In addition, we will discuss potential field-based methods to determine how different resistance levels (low, moderate, high) impact mosquito control outcomes, what we can learn from agricultural pest control, and what additional information is needed to understand the link between mosquito resistance monitoring and management.

135 **NEVBD's pesticide resistance program: a collaborative system for monitoring, assay development and education.**

Lindsay Baxter, lb694@cornell.edu

The Northeast Regional Center for Excellence in Vector-Borne Diseases (NEVBD) pesticide resistance monitoring program was developed in 2019. The goal of the program is to support and expand capacity across the Northeast and Mid-Atlantic regions to collect baseline resistance data and monitor emerging resistance in mosquito populations. The NEVBD has developed a specimen submission and testing system, adult topical, larval bioassays and mechanism testing in response to regional needs. In addition, we have conducted onsite resistance monitoring training and developed materials to assist municipalities in collecting and testing mosquitoes. From 2019-2022 agencies directly submitted *Aedes albopictus* and *Culex pipiens* for larvicide and adult bioassay testing. Mosquitoes were submitted from 11 of 13 states in NEVBD's catchment area including larval assay requests from 26 agencies spanning 8 states and adult bottle bioassay requests from 20 agencies spanning 9 states. NEVBD and partnering agencies have identified moderate-high resistance to pyrethroids in several locations and emerging resistance to organophosphates in the mid-Atlantic coastal region. In 2022 we embarked on a more thorough investigation of *Cx. pipiens* pyrethroid and organophosphate resistance in the lower Chesapeake Bay area.

136 **Crouching Tiger, Obvious Trouble: Resistance Management in Miami-Dade County**

Isik Unlu, isik.unlu@miamidade.gov, Miami Dade County Control, Chalmers Vasquez, Aimee Cabrera, Yanet Chiong, Joseph Blackman, Pedro Errasti, William Petrie, Eva Buckner

Culex quinquefasciatus Say, member of the *Culex pipiens* complex and commonly known as the southern house mosquito, is present in the Americas, Australia, Asia, Africa, Middle East and New Zealand. This species, considered a nuisance due to their aggressive and nocturnal biting behavior, is also a vector of several pathogens, including the *Wuchereria bancrofti* (bancroftian filariasis) and the West Nile virus. *Aedes aegypti* (Linnaeus), commonly known as the yellow fever mosquito, is an important vector of arboviruses such as dengue, chikungunya and Zika. *Cx. quinquefasciatus* and *Ae. aegypti* are both abundantly present in Miami-Dade County. In 2020, West Nile Virus became increasingly prevalent resulting in 60 WNV human cases. In addition, the County received 491 referrals from the Florida Department of Health, for suspected cases of dengue, chikungunya and Zika as of September 20, 2022. Eighteen confirmed local cases of Dengue have been reported. Control of the mosquito species that spread these viruses can be done through the use of wide area adulticide applications using truck-mounted sprayers. The efficacy of these treatments is highly dependent on the insecticide susceptibility status of the local mosquito populations. Miami-Dade Mosquito Control in partnership with UF/IFAS Florida Medical Entomology Laboratory has been conducting insecticide resistance monitoring on adult mosquitoes since 2020. *Cx. quinquefasciatus* and *Ae. aegypti* eggs from 52 different locations were tested. Bottle bioassay results showed 0 to 51% mortality from pyrethroids for *Cx. quinquefasciatus* while organophosphates showed 76 to 100%. Bottle bioassay results showed 0 to 55% mortality from pyrethroids for *Ae. aegypti* while organophosphates showed 100%. Based on their individual flight characteristics, a GIS layer of buffer regions was mapped around known active sites: 1mile buffer for *Cx. quinquefasciatus* and 0.2 mile buffer for *Ae. aegypti*. This was done to coordinate adulticiding applications based on resistance data.

Using NASA Satellite Data to Complement Vector Control Practices Symposium

137 Improving Vector Control with NASA Data: An Overview of NASA Health and Air Quality Applications

John Haynes, jhaynes@nasa.gov

Climate change, urbanization trends, and agricultural practice alterations influence the spread of vector-borne diseases, which affect more than half of the global population. Integrated vector control management is key to bridge disciplines, identify knowledge gaps, and develop best practices for mitigating vector-borne disease risk and spread among global populations. One innovative perspective includes Earth-observing satellites, which collect information on the dynamic state of environmental parameters important to public health. These data complement traditional epidemiological tools and allow for a new point-of-view in analysis and interpretation of spatial and temporal data used in early warning systems for diseases. This presentation will present an overview of the NASA Health and Air Quality Applications focus area. In particular, it will offer a closer look at selected applied research projects that use NASA satellite data to assess mosquito habitat suitability and disease risk. This information strengthens intersectoral collaborations that incorporate satellite- and ground-based data in vector control management, aiming to positively impact health outcomes. Session attendees will

learn about the program's activities and opportunities including, but not limited to, participation in upcoming NASA data trainings and the GEO Health Community of Practice network.

138 Blending Multi-source Data Sets to Forecast Vector-borne Disease Risk

Assaf Anyamba, Anyambaa@ornl.gov

Vector-borne and zoonotic pathogens comprise a substantial portion of the global disease burden causing ~1.4 million deaths annually, and account for approximately 17% of the entire disease burden caused by parasitic and infectious diseases. Current Public Health and Department of Defense surveillance systems track individual infectious disease cases to report disease trends across populations, but these are retrospective and do not provide predictive information that could identify high-risk areas to better public health as well as deploying military personnel. Epidemiologies of many vector-borne pathogens are driven by climate and environmental conditions that critically influence vector survival, reproduction, biting rates, feeding patterns, pathogen incubation and replication, and the efficiency of pathogen transmission among multiple hosts. Specific patterns of climate and weather anomalies preceding increased vector populations and resulting disease outbreaks are extensively documented for dengue, chikungunya, Rift Valley fever, and plague. To address this gap in early warning and response to vectorborne disease threats, we are designing a unified application that utilizes multi-decade satellite derived measurements of climate variables map and forecast the risk of various diseases including chikungunya, dengue, Rift Valley fever and hantavirus. To design such an integrated system, we are (1) compiling historical outbreak data from various sources including ProMED and PAHO, (2) global climate data from NOAA and NASA including rainfall, land surface temperature, (3) Population data from the Socioeconomic Data and Applications Center (SEDAC) and LandScan Population database from ORNL, (4) Various mosquito vector distributions from VectorMap (Walter Reed Biosystematics Unit) and VectorBase (National Institute of Allergy and Infectious Diseases (NIAID) Bioinformatics Resource Center (BRC). We plan to utilize various machine learning methods to combine these complex data sets to map and forecast on a monthly to seasonal basis the risk of various disease threats globally.

139 Making sense of disparate data types using VectorSurv

Christopher Barker, cmbarker@ucdavis.edu, Tim Valdepena, Jody Simpson, Shawn Ranck, Kurt Johnson, Lincoln Wells, Christina De Cesaris

VectorSurv is a publicly-funded, online interactive software system that provides tools for real-time collection, visualization, and analysis of data on vectors and vector-borne pathogens. The system addresses urgent needs for data modernization in the U.S. to deal with the growing threats of mosquito and tick-borne diseases. The system was initiated in 2006 to provide decision-support tools for West Nile virus and later to inform surveillance and control strategies for invasive *Aedes aegypti* and *Aedes albopictus*. VectorSurv now serves > 170 vector control and public health agencies in more than 18 U.S. states and territories, and the system is immediately scalable to any U.S. jurisdiction. Following CDC's national framework for prevention and control of vector-borne diseases, VectorSurv provides interconnected, quality data to inform decision-makers at all levels – local, state, and national. This presentation will provide an update on VectorSurv's online calculators and data visualization tools. These tools enable decision-makers to view and analyze near-real-time data to understand how vector populations and arbovirus risk respond to control efforts and environmental conditions.

Sarah Wheeler, swheeler@fightthebite.net

The Sacramento-Yolo Mosquito and Vector Control District (Sac-Yolo MVCD) manages mosquito populations across urban, suburban, and rural landscapes to protect the public from mosquito-borne diseases and nuisance biting. To make control decisions a network of surveillance systems including: mosquito traps, dead birds, sentinel chickens, larval dipping, and molecular surveillance for mosquito-borne viruses are utilized. The data collected from these systems are analyzed and responses are initiated. A response can range from setting a mosquito trap to applying a larvicide or potentially deploying an airplane for an adult mosquito control mission. Data analysis tools such as infection rates and vector index are critical for interpreting risk and measuring the efficacy of control interventions. The forecasting and monitoring of weather, including wind speed and direction and air temperature, is critical for conducting effective control applications. Additionally, the district monitors potential larval habitats including the intentional and predictable movement of water onto pastures, fields, and wetlands, and the unpredictable pooling of water that can have many sources including beaver dams, seepages, and fresh water tidal flooding. These pools of water can support the development of immature mosquitoes and thus are identified, surveilled for mosquito larvae, and treated with larvicides and/or mosquitofish as necessary to prevent emergence of adult mosquitoes. The surveillance tools used Sac-Yolo MVCD will be reviewed, typical responses to surveillance metrics presented, and areas for potential improvement discussed.

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Successful vector control depends not only on the effectiveness of the control agent but also on the efficiency of the delivery system and degree of achieved coverage or impact on the vector population, either by impinging elevated mortality or reduced fecundity. Typically, vector population suppression should be over 80% to significantly reduce pathogen transmission and disease. Assuring effective coverage involves precise understanding of the spatial and temporal distribution of the vector population and human infections. Insect vectors and disease cases show over dispersed distributions, where 70-80% of the individuals occur in clumps or aggregations, also referred to as hot spots. Georeferenced data within a Geographical Information System (GIS) allows identifying hot spots and understanding the environmental variables associated with them. Data derived from satellite images, such as landscape elements (vegetation, impervious surface, water bodies, elevation etc.) and climate (e.g., temperature, precipitation) are essential variables to describe and understand the dynamics of the vector population and pathogen transmission. For example, urban heat islands, which are areas with higher surface temperature than undisturbed surroundings, have been associated with elevated *Aedes aegypti* populations and dengue transmission. Similarly, vector abundance and spatial distribution usually vary along environmental gradients. Having such a GIS in place allows planning the deployment of surveillance tools, vector control measures, and evaluating the impact of control. Surveillance activities are greatly improved by using electronic devices (cell phones, tablets) to capture data in the field, which could be monitored in real time to improve supervision and quality control. The progress of the activity can then be visualized in dashboards that can be shared with stakeholders. Some examples are provided in the presentation.

West Nile virus and other arthropod-borne pathogens in Arizona: Surveillance and mitigation strategies Symposium I

143 Preventing the spread of Rocky Mountain Spotted Fever (RMSF) in Arizona

John VandenBrooks, john.vandenbrooks@gmail.com

Rocky Mountain Spotted Fever (RMSF) is one of the deadliest tick-borne disease in the United States with a mortality rate as high as 80% if not treated in the first five days. During a recent outbreak of RMSF in Arizona, the brown dog tick (*Rhipicephalus sanguineus*) was identified as a novel vector for the causative bacteria (*Rickettsia rickettsii*). However, while the brown dog tick is present in every state in the U.S., it is only known to vector RMSF in the Arizona region. The RMSF case rate has risen year over year and a recent outbreak in Mexico has also tied to *R. sanguineus*. Given the apparent spread of the vectoring capabilities of *R. sanguineus*, it is critical to develop strategies to predict and prevent future RMSF outbreaks. To this end, our team has taken a One Health approach to answer the question of why the brown dog tick can act as a vector for RMSF in the Arizona region, but not elsewhere.

Our results show that the spread of RMSF in Arizona is due to four factors: (1) the presence of a distinct population of brown dog ticks, (2) variation in the genetic strain of the causative agent *R. rickettsii*, (3) canine seroprevalence rates, and (4) geographic and climatic factors. We have identified three genetically distinct clades of ticks that vary in the *R. rickettsii* infection rates. In sequencing intergenic regions of *R. rickettsii*, we identified variation in the rickettsial strain. Using immunofluorescence assays, we determined that canine seroprevalence rates are rising and seropositive dogs were found in regions that have previously not reported any human cases. Even more concerning, 12 of 16 counties surveyed were at medium to high risk for outbreak. Together, this multi-factorial model is a first step towards predicting and preventing future RMSF outbreaks.

144 Ceratopogonid midges as potential vectors of *Onchocerca lupi* in Northern Arizona

Chandler Roe, chandler.roe@nau.edu, Kelly Upshaw-Bia, Olivia Holiday, Jennifer Urbanz, Guilherme Verocai, Chase Ridenour, Morgan Ford, Theodore Kennedy, Crystal Hepp, Jason Sahl

Onchocerca lupi (Rodonaja, 1967) is an understudied vector-borne filarial nematode that causes ocular onchocercosis in dogs, cats, coyotes, wolves, and recently, humans. Onchocercosis in dogs has been reported with increasing incidence worldwide. However, despite the growing number of reports describing canine *O. lupi* as well as global zoonotic infections, the geographic distribution, prevalence, and vector species of this parasite remains largely unknown. Here, our study aimed to identify the occurrence of *O. lupi* infested dogs within the Navajo Reservation which spans both northern Arizona and New Mexico, USA. Utilizing these results, targeted insect trapping was performed to identify the vector of this nematode. This targeted and controlled sampling of both host and vector provide novel insights regarding the occurrence and potential endemic range of *O. lupi* in the United States. Furthermore, the identification of vectors in close proximity to human populations coupled with multiple, local zoonotic cases highlights the one health importance of *O. lupi*.

145 **Evolutionary ecology of kdr resistance mutations in *Aedes aegypti* mosquitoes**

Silvie Huijben, shuijben@asu.edu, Brook Jensen, Rachel Althoff, Joshua Kalmouni, Joshua Ain, Chance Spurlin, Krijn Paaijmans, Alden Estep

Mosquito vector control relies heavily on insecticides to control or prevent the (re)emergence of vector-borne diseases, yet the efficacy of insecticides is greatly limited by widespread resistance to the active ingredient. Insecticide resistance is an evolutionary problem at the core. To design optimal insecticide resistance management (IRM), a deep understanding of the evolutionary ecology of mosquito populations is essential. For this, we need to measure both the fitness benefits in the presence of insecticides at different dosages (phenotypic resistance), and fitness costs in absence of insecticide of resistance mutations. To establish phenotypic resistance, we compared the variability in mortality between different bioassays that measure insecticide resistance: CDC bottle bioassay, WHO tube test, and topical application bioassay. This study showed that topical application bioassays performed best by introducing the least amount of variability in mortality assays. In contrast, the CDC bottle bioassays introduced a high level of variability. Next, we compared the frequency of kdr resistance mutations (V1016I and kdr F1534C) in the *Ae. aegypti* population in Maricopa County in recent years, showing an increase in kdr resistance over time. Finally, we measured the fitness benefits and fitness costs of various homozygous and heterozygous kdr genotypes using lab crosses at different insecticidal pressures and different ambient temperatures.

Overview of Applied Research Programs at the District/Program Level Symposium II

146 **SLCMAD! Where Research Ideas Connect**

Christopher Bibbs, chris@slcmad.org, Greg White, Ary Faraji

Building capacity and expertise at Salt Lake City Mosquito Abatement District to implement applied research and become a continuously learning agency. This talk will be a brief canvas of how SLCMAD has grown to accommodate its applied research goals, specifications for the new facility with it's intent to host collaborators, and programmatic growth with local Universities to develop pipelines for research labor. District change has been enacted successively through the executive director (budget, infrastructure, ideation), assistant director (program development, resource management), and laboratory director (personnel management, growth opportunities) at SLCMAD. An exposé on successful research based internships and continuing projects will also be shown.

147 **Designed with the Desert in Mind – A Research Program for Coachella Valley MVCD**

Jennifer Henke, jhenke@cvmosquito.org

The Coachella Valley Mosquito and Vector Control District (the District) has had a long partnership with universities, government agencies, and industry partners to examine mosquito and vector control within its unique environment. With extreme temperatures in the summer and very little precipitation, long-term control strategies need to withstand long days of sunshine and sometimes high wind in the evenings. District staff have found that solutions designed for other agencies sometimes

need to be adjusted to work within the desert environment. The District maintains a research fund to support university and government researchers to develop studies that will improve the District's efficacy in controlling mosquitoes and other vectors. We also have actively participated in the region's workforce development programs, partnering with agencies who are training for healthcare careers from high school and beyond. Here, I will describe the organization of our research program, the balancing of requests to partner for projects while maintaining the priorities of serving communities, and issues to consider for those looking to develop their own programs.

148 An Overview of the Applied Research Program at Anastasia Mosquito Control District

Whitney Qualls, wqualls@amcdf.org

Anastasia Mosquito Control District (AMCD), St. Augustine, Florida has collaborated with universities, institutes, and industries to investigate and develop new methods/techniques to improve customer service, arbovirus and mosquito population surveillance, and operational control. These collaborative projects and their accomplishments have benefited AMCD's operational programs. AMCD has collaborated with industry partners to evaluate, modify, and improve the efficacy of several different types of surveillance and control traps, attractants, repellents, insecticides, and equipment. The district built a wind tunnel / olfactometer laboratory, a droplet laboratory with a laser machine to characterize insecticide sprays, 24 field larvicide evaluation pools, three large outdoor enclosures, a quarantine laboratory with two green houses, bioassay laboratory, and a molecular laboratory for applied research. These laboratory conditions and facilitates promote and benefit applied research and allow the district to be competitive for several grants from federal, state, and industry partners. The district trains 6-7 visiting scientists world wide, 7-8 intern students, and generates 10-15 applied research articles annually. Currently, AMCD has 5 Ph.D. Scientists and 5 M.S. Biotechnicians included 1 Ph.D. student (through the University of Florida) in the team with 2 Federal grants, 3 State grants, and 5 industry funded projects. In addition, the Disease Vector Education Center (6,000 s.f.) and the SIT mass rearing facility (6,000 s.f.) are under construction and expected to be completed by the end of 2022. Since 2004, the district began an applied research program, enhanced employee training and public education, and adopted new technology and innovation that have improved operation's efficiency and saved millions of tax payers money.

149 Overview of applied research at the Metropolitan Mosquito Control District

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The Metropolitan Mosquito Control District is located in Minneapolis & Saint Paul (MN) and provides services to the three million people in the seven-county metropolitan area. The District's primary mission is to provide vector and annoyance control of mosquitoes and black flies. In addition, the District monitors tick populations to educate and reduce citizens risk of tick-borne illnesses.

The District is an independent taxing district and totally supported by public funds. Therefore, research is primarily focused on the betterment of District operations or on topics that directly affect those services. The District is advised by a Technical Advisory Board and multiple research projects have been the result of their recommendations. Most of our applied research is self-generated by our various work teams to answer internal questions and used to increase our operational effectiveness and improve

efficiency. The District has previously conducted public surveys and annoyance tolerance studies to better understand our citizens to help direct our program.

West Nile virus and other arthropod-borne pathogens in Arizona: Surveillance and mitigation strategies Symposium II

150 Detection and characterization of a novel insect-specific flavivirus in a Maricopa County *Aedes aegypti* mosquitoes

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The *Aedes aegypti* mosquito is infamously known to transmit pathogenic viruses including Zika (ZIKV), Dengue (DENV), and Chikungunya (CHIKV). While mosquito surveillance efforts in Maricopa County have not detected local circulation of these pathogens, sporadic outbreaks of Dengue and Zika virus in the border region are cause for concern. The lack of local circulation in Arizona, despite a long-established competent vector, has led us to wonder if there are mechanisms in place that may inhibit local circulation.

In this project, we screened over 100 mosquito pools that were geographically distributed throughout the 25 vector districts of Maricopa County using a pan-flavivirus assay optimized for next-generation amplicon sequencing purposes. Although we did not detect flaviviruses that are pathogenic to humans, we found 35 pools positive for the presence of a novel and uncharacterized flavivirus. As of September 2022, there are 21 known flaviviruses thought to only infect insect hosts, including mosquitoes, ticks, and sandflies. A growing body of research suggesting that insect-specific flaviviruses may competitively inhibit their pathogenic counterparts. This project represents an effort to learn more about an insect-specific flavivirus that could influence *Ae. aegypti*-vectored viruses of concern.

151 Mitochondrial population phylogenomics of the West Nile virus vector *Culex quinquefasciatus* in the southwestern United States

Zachary Barrand, zbarrand@tgen.org

Since the introduction of West Nile Virus into the United States in 1999, the Centers for Disease Control and Prevention have reported over 51,000 cases of WNV disease, 30% of which have been noted within the southwestern United States. West Nile Virus is an enzootic pathogen that spills-over into human populations when humans come into contact with infected mosquitoes. The primary vectors of WNV, *Cx. quinquefasciatus* and *Cx. pipiens*, are vital to the maintenance and transmission of WNV. Although the genetic structure of *Cx. pipiens* complex populations have been extensively studied around the world, little is known about the genetic composition of *Cx. quinquefasciatus* populations, within Arizona, the surrounding southwestern United States, and associated implications for WNV circulation. To better understand *Cx. quinquefasciatus* populations in the region, we developed a long-range PCR assay, sequencing strategy, and bioinformatics pipeline targeting whole mitochondrial genomes. These tools have helped us to better understand population clusters and structure over time and space within and across pooled samples obtained during routine surveillance activities conducted by Vector Control Division of Maricopa County Environmental Services and Coachella Valley Mosquito and Vector Control District.

152 **Comparative West Nile virus surveillance and mitigation strategies in Maricopa County**

John Townsend, john.townsend@maricopa.gov

West Nile virus and other arthropod-borne pathogens in Arizona: Surveillance and mitigation strategies. Comparative West Nile virus surveillance and mitigation strategies in Maricopa County.

In this presentation we will compare the surveillance and mitigation efforts of the contrasting years of 2021, where Maricopa County, Arizona, experienced the largest West Nile Outbreak in the United States and the current 2022 season, where positive Saint Louis Encephalitis mosquito samples are dominating surveillance numbers. Saint Louis encephalitis reemerged in Maricopa County in 2015 and we have seen concurrent infections in our mosquito populations, since the re-introduction of the virus. We will discuss and compare the mitigation efforts in 2021 and the current mitigation efforts of 2022. We will look at surveillance efforts between the years and track the current and previous positive mosquito samples on a week by week basis. We will also compare human cases between the two seasons and track cases throughout each CDC epi week.

153 **The Persistence of West Nile Virus in the Southwestern United States**

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Since the first detection of West Nile virus (WNV) in the US in 1999, nearly 55,000 people have tested positive and more than 2,700 have died. Over the past decade, Arizona has ranked 3rd for highest total and neuroinvasive disease cases, with the majority occurring in Maricopa County. While 2021 was an average year for WNV cases across much of the US, AZ faced its highest number of cases ever, with Maricopa County residents comprising ~86% of those cases. At the onset of this study in 2016, we hypothesized that the county likely experienced repeated introductions year after year, and that given the distance between several southwestern counties that frequently report human cases (eg. Riverside, Clark, Washington, and Yuma counties), outbreaks were geographically distinct. To address our hypotheses, we've partnered with vector control agencies throughout the southwest, who have provided more than 800 WNV positive samples that we sequenced using a novel tiled amplicon sequencing approach. Our study reveals novel insights regarding West Nile virus in Maricopa County and the greater southwest: 1) WNV is endemic in Maricopa County, overwintering and reemerging annually, with a limited number of new and short-lived importations, 2) This endemic WNV population is the longest known in any US county, persisting over the past decade, 3) Preliminarily, that genomically-derived effective population size estimates are strong predictors of spillover risk, and 4) WNV in Maricopa County repeatedly spills over into other southwestern counties, indicating that this viral population is not only important for the public health of Maricopa County residents, but the rest of the region as well. Genomic results have been made publicly available, to provide situational awareness to our partners and the public:

<https://nextstrain.org/community/HeppLab/WestNileVirus@main/NorthAmerica?c=county>

Resist the urge to panic: Managing resistance in field operations Symposium II

154 Resistance breeds creativity: How IR redefined our thresholds, timings, and treatments

Nicholas DeLisi, ndelisi@stpmad.org, Lisa Rowley, Kevin Caillouet

St. Tammany Parish Mosquito Abatement District (Slidell, LA) has measured resistance in *Culex quinquefasciatus* to pyrethroids and organophosphates. As a result, we created a mosquito management plan (<https://stpmad.org/mosquito-management-plan>) sensitive to applying selective pressure and the development of insecticide resistance. Our intent is to retain susceptibility to insecticides currently in use while still maximizing treatment efficacy despite field-relevant resistance. Topical bioassays and enzyme assays inform the presence of resistance and dictate which active ingredients are used in rotation, as well as their respective application rates. Treatment thresholds linked to West Nile virus (infected pools, mosquito abundance, and other indicators) are reevaluated annually to decrease the likelihood of repeat-applications within the same class of insecticides. Finally, we lengthened the time between applications within the same insecticide class to alleviate selective pressure in the short-term. While resistance has challenged our day-to-day operations, it has also provided an opportunity to refresh our management plan.

155 Country-wide scope of pyrethroid resistance in *Aedes aegypti* in Peru and implications for resistance management

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Aedes aegypti reinvaded Peru in 1984, with initial detection in Amazonian states near Brazil and recent establishment in southern states near Chile. More than 144,000 cases of dengue have been reported in 2017-2020. Collaborative vector surveillance work with local Ministry of Health and Military units allowed collection of 2,400 *Ae. aegypti* from 55 locations in coastal and jungle states from 2017 to 2022. Specimens were tested for knockdown resistance (*kdr*) mutations V1016I and F1534C. Mosquitoes from northern coastal and four northern jungle states were fixed or nearly fixed ($\geq 95\%$) for 1534CC; 1534FF mosquitoes (29-100%) were found in southern and one northern jungle states. Moderate to high levels of 1016II were found in northern coastal states (20-86%); low to high levels of 1016II were found in northern jungle states (0-89%); and low to moderate levels of 1016II were found in southern states (0-24%). Eight genotypes were observed (IICC, IIFC, VICC, VIFC, VIFF, VVCC, VVFC, VVFF) with resistant genotypes (VICC, IICC) indicative of higher resistance (20X-60X), recorded in northern states, and less resistant genotypes (VVCC, VVFC, VVFF) indicative of lower resistance (4X) in southern states. Our results showed that *kdr* mutations are broadly distributed in Peruvian *Ae. aegypti*, yet with considerable variation even within close geographic proximity. A higher proportion of more resistant genotypes were found in northern states, which have been subject to many years of control efforts, than in southern regions, more recently colonized locations or locations with little vector control activity. This may explain decreased effectiveness of pyrethroid insecticides for *Ae. aegypti* control in northern Peru. Phenotypic and biochemical responses of northern jungle *Ae. aegypti* to pyrethroids will further validate the use of these genetic markers as a rapid insecticide resistance screening tool. This study provides insights into pyrethroid resistance mechanisms and may guide mosquito control operations.

156 **Operational perspectives of mosquito larvicide resistance**

Tianyun Su, stevensu1995@gmail.com

Often mosquito control is the most effective or the only intervention to mitigate mosquitoes and mosquito-borne diseases. In mosquito control operations, larviciding to target most aquatic stages with limited distributions by biorational larvicides is more cost-effective. However available environmentally friendly larvicides are quite limited now due to numerous reasons, among which development of resistance in target species should never be overlooked. Risk of resistance development and associated cross-resistance in response to repeated sublethal exposure varies among the larvicidal active ingredients with different modes of action. In mosquito control operations, prevention of resistance evolution in the first place and susceptibility restoration after resistance occurrence are the primary strategies to ensure the sustainability of control efficacy. Tailor-made resistance management tactics such as withdrawal of treatment, application in mixture or rotation can be developed and implemented after confirmation of resistance occurrence in target species by ruling out factors from products, habitats, and applicators.

157 **Resistance vs. tolerance: evaluating the potential for larvicide resistance in the northeast US**

Joseph McMillan, josmcmil@ttu.edu, Michael Olson, John Shepard, Tanya Petruff, Philip Armstrong

The recent emphasis on understanding and identifying larvicide resistance in mosquito populations has resulted in a nationwide investigation of product susceptibility profiles. One striking result from these studies is the broad range in observed lethal doses for active ingredients (AI) in different mosquito species. Is this evidence of resistance to or tolerance of the product? We use results from *Lysinibacillus sphaericus* dose-response studies, observations of mosquito control operations, field experiments, and in-lab assays performed in Connecticut, U.S. to make the argument that larvicide dosage relationships can be species-specific and are, in some cases, indicative of AI ineffectiveness or tolerance. Specifically, we show that *L. sphaericus* is highly lethal to *Culex pipiens* mosquitoes in both controlled and applied settings while *L. sphaericus* is ineffective for container breeding *Aedes* spp., such as *Aedes albopictus* and *Aedes triseriatus*, as well as *Culiseta melanura*. While these results demonstrate the proof of principle for *L. sphaericus* specificity in *Culex* spp. mosquitoes, further research is needed to identify the chemical, physiological, and molecular basis for such species-specific relationships as well as strategies for effective control of non-*Culex* or mixed genus larval habitats.

158 **What leads to pyrethroid resistance in *Culex tarsalis*?**

Tara Thiemann, tthiemann@pacific.edu, Billy Mortola, Sumiko De La Vega, Bonnie Ryan

Culex tarsalis is one of the most abundant vectors of encephalitis viruses in California. Pyrethroid insecticides are crucial in reducing mosquito populations and thus the transmission of vector-borne pathogens. Over the last several years, there have been increased reports of insecticide resistance in *Cx. tarsalis*. Several graduate students in the Thiemann Lab have worked to characterize the mechanisms of this resistance at multiple sites across Northern California. Data analysis is ongoing, but it appears that both a target-site genetic mutation and increased enzyme levels are contributing to

Cx. tarsalis pyrethroid resistance. Here, we will look at which resistance mechanisms seem to be most important.

159 **Monitoring of resistance in Aedes aegypti population from different habitats against different insecticides**

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Mosquitoes are known as vectors of many life threatening diseases like malaria, filariasis, Japanese encephalitis, chikungunya and dengue fever. In tropical countries, over 2 million people are at risk of mosquito borne diseases. Mosquito can transmit more disease than any other group of arthropods. Among all, Aedes aegypti are becoming the most important vector of many viruses such as Dengue virus. Different chemicals are being used to control mosquitoes. Due to blind use of chemicals, insecticidal resistance is developing in mosquitoes. In this study, we evaluated four different insecticides (alpha cypermethrine, Bti, lambdacyhalothrin and temephos) with following concentrations (alpha cypermethrine: 11, 1.1, 0.11, 0.011, 0.0011 ppm), temephos: 7, 0.7, 0.07, 0.007, 0.0007 ppm), (lambda cyhalothrin: 100, 10, 1, 0.1, 0.01 ppm), (deltamethrin: 3, 0.3, 0.03, 0.003, 0.0003 ppm), (Bti: 10, 1, 0.1, 0.01, 0.001 ppm) were applied on different groups of Aedes aegypti larvae. Each treatment was consisted of four replications with control group (Source water). The data was recorded after 12, 24, 48, and 72 hours of exposure time. The results showed that deltamethrin was the most effective and lethal against larvae of Ae. aegypti while Bti found least effective. Higher percentage of mortality by these insecticides recorded in rural areas and this percentage decreased as the urban mosquito population assayed with the same pesticides. The analysis of following enzymes (Oxidases, Mixed function enzyme, Glutathione S- Transferase, Acetylcholine esterase) also showed high level of resistance estimated in population from agricultural farms followed by urban areas. While low/ minimum level of resistance in remote rural areas.

160 **Guidelines for Larvicide Resistance Testing: Applications, Challenges and Modifications**

Laxmi Shanmugam, lshanmugam@clarke.com

Guidelines for Larvicide Resistance Testing: Applications, Challenges and ModificationsAs the use of larval control methods increases, so does larvae resistance to commonly used active ingredients (AI) and their modes of action (MOA). Being able to evaluate and monitor larval resistance to insecticides through the World Health Organization (WHO) cup bioassay is crucial for responsible stewardship and product rotation to combat resistance and extend the useful life of this vital mosquito control tool. This session will highlight the need to conduct larvicide resistance testing, delve into the mechanisms of resistance (MOR) that different MOAs present and introduce ongoing testing in Clarke's labs on four proposed WHO cup bioassay modifications aimed at improving its overall utility and reliability. These continuing modifications will include modifying test methods to better mimic environmental conditions where larval products are used in the field for mosquito control; delivering improved test result accuracy that reflects product efficacy; applying test results findings to understand better the mechanism of resistance observed; and proposing solutions for managing larvicide resistance in mosquito operations. The goal of this session is to contribute to the ongoing discussion on resistance evaluation by highlighting the need for improved larvicidal resistance testing, gaining a better understanding of MORs and how they mutate in relation to MOAs, and sharing current progress with implementing test modifications.

Spatial Repellents Symposium

161 **Spatial Repellents: Current Status and Potential Future in Mosquito/vector Population Management Strategies**

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Recently there has been an increased emphasis on adding new tools to our mosquito management toolbox due to an increase in the development of insecticide resistance, a lack of new pesticide products, and a reluctance to use topical repellents. Spatial repellents have been suggested as a potential tool. This presentation will provide an introduction to a symposium which will provide an overview of the current status and potential future role that spatial repellents might play in future mosquito/vector population management strategies. A brief history of spatial use will be provided. This will include current methods for evaluating candidate compounds/products under laboratory, semi-field and field conditions. Current concepts of what constitutes a spatial repellent and a generalized definition for this category of compounds will be provided. While synthetic pyrethroids have been used in many currently available commercial products, there will be a presentation on the potential future use of essential oils as spatial repellents.

162 **The Surprising Intersection of Custom Flight Chambers, Quantitatively Gas Chromatography and Tissue Engineering in Mosquito Research and Spatial Repellents**

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Volatile (i.e., airborne) spatial repellents are powerful tools in the battle against mosquitoes and the diseases they vector. Ideally, these airborne compounds repel mosquitoes and reduce biting/blood-feeding behaviors—all at nonlethal concentrations—thereby decreasing disease transmission without driving resistance selection. Our research therefore focuses on developing benchtop-based platforms to quantitatively define minimum threshold airborne concentrations of spatial repellents (e.g., transfluthrin) that alter mosquito flight and host-seeking behaviors as well as the reproductive impacts of such exposures. Recently we showed that standing, overlaid airborne concentration gradients of host cues (CO₂ and BG-Sweetscent) and the volatile spatial repellent transfluthrin (5× and >10×, respectively) can be established in an all-glass wind tunnel/flight chamber of our design. Further, *Aedes (Ae.) aegypti* females in these environments had both reduced flight and movement activity as quantified by videography. We have also recently developed an engineered model human skin tissue, complete with a microvascular bed and blood, and demonstrated that *Ae. aegypti* bite and blood feed naturally on these constructs. We aim to converge these efforts into unified platforms for comprehensive assessment of spatial repellents. Here we will overview our unique program that sits at the nexus of custom mosquito flight chambers, active air sampling, quantitative gas chromatography-mass spectrometry and tissue engineering.

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Bayer Cropscience LP. These entities did not support this work in any way and had no role whatsoever in this research. BJW is an inventor on US patent 7,601,525 and application 20180078423 (PCT/US2016/029122).

163 3D printed transfluthrin based novel spatial repellent devices for enclosed spaces

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Christopher Batich, Daniel Kline

Mosquitoes transmit various pathogens to humans and animals, which may result in a variety of diseases. The need for newer repellents and their release mechanisms have increased due to development of insecticide resistance and reluctance by many to use traditional topical repellents. Sustained release spatial repellents have been tested and have shown promise in preventing vector-host contact in small, enclosed areas such as personal space and tents. Sustained release devices have been designed to provide long term repellency over extended periods of time by deploying a set of devices. These devices have been evaluated under semi-field conditions for their efficacy when used at tent entrances to provide personal protection. Free flying mosquitoes were released and potentially recaptured using a baited trap set inside the tent as a surrogate host to determine the repellency. Mosquito bioassays were conducted inside the tents to determine the spatial distribution of the spatial repellent within the tent and the efficacy of the devices to affect mosquito behavior. This ensures that a sufficient amount of active ingredient was being released and spatially distributed in a way to affect the host seeking and host location behaviors of any free fliers which pass through the array of devices located at the tent entrance. These novel sustained release transfluthrin devices have been proven efficient up to 6 weeks in reducing the number of mosquitoes from successfully locating a potential host.

164 Evaluating natural product repellency in a small-tube laboratory assay

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Spatial repellents are fast becoming a product class that allow for rapid protection from blood-feeding arthropods because of their relative ease of use and safety to users. However, already widespread insecticide-resistant pest populations (resistant to pyrethroids, in particular) may be less susceptible to some of these products. Natural products represent a potential source of new control tools, as many of these naturally derived compounds are structurally diverse and are efficacious themselves or represent leads to better synthetic derivatives. We screened a variety of natural products in a laboratory spatial repellency assay on *Aedes aegypti* mosquitoes. Repellency and toxicity dose-response curves were obtained for all compounds screened, and these curves were used to compare the relative efficacy of compounds to current commercially available spatial repellents. A number of natural products were capable of producing knockdown and toxicity at 1 hr and 24 hr respectively. Overall however, they were considerably less toxic than most synthetic repellent molecules on the market today. In terms of repellency, natural products were segregated into clear categories based on their efficacy. An analysis of the physicochemical properties of the most successful chemistries was also performed. These results demonstrate the potential of using natural products as future spatial repellent products and highlight select compounds and physicochemical properties within this class, which may be the most promising for future study and development.

Volatile Pyrethroids Against Mosquitoes: A meta-analysis of the association between mosquito exposure to volatile pyrethroid-based spatial repellents and mosquito bite prevention

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In recent years, products using volatile pyrethroids have been of increasing interest for addressing outdoor-biting Anopheles mosquitoes responsible for malaria transmission, as well as dengue virus and Zika, both of which are untreatable and on the rise due to rapid urbanization. There is a need to harmonize the growing evidence around volatile pyrethroids against mosquitoes, for the existing evidence base is heterogeneous comprising of different active ingredients, prototypes, study methodologies and scenarios, and entomological outcomes. This meta-analysis study (PROSPERO #CRD42021268852) seeks to consolidate this evidence base, summarizing their protective efficacy using human landing catch and/or trap density as primary outcomes. Secondary outcomes include their duration of efficacy, entomological outcomes such as knock-down, delayed mortality, fecundity and oviposition, and diversion. Results are stratified by active ingredient, spatial repellent format, mosquito species affected, indoor vs outdoor studies, field vs semi-field study, and insecticide resistance assessments among field-caught mosquitoes.

We present interim results on this project, including a literature search of 1,145 published abstracts to identify 42 full-text articles eligible for inclusion, and acquisition of the majority of original datasets from these publications. Data synthesis includes the creation of forest plots summarizing the protective efficacy of volatile pyrethroid products and prototypes from each study, as well as preliminary analyses on aggregate data summarizing the overall protective efficacy of various product classes and prototypes separated by the variables described for stratification above, with separate plots for each entomological outcomes on post-exposure effects where data are available. Sensitivity analysis investigates the impact of temperature and relative humidity on their duration of efficacy, drawing upon satellite data as necessary. Results are expected to be useful for parametrizing disease transmission models, and to determine settings and use cases where these products can most effectively reduce disease transmission.

Evaluation Systems for Spatial Repellents against Ticks

Muhammad Farooq, mfarooq@amcdfl.org, Rudy Xue

Spatial repellents are now becoming 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 determine the worthiness of the repellents. These evaluations against ticks are carried out either to assess efficacy of AI, the effectiveness of 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 vapors generated by a personal mosquito repellent device were 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% adults moved away from repellent compared to 27 % in control. These numbers were 43 and 20 % for nymphs.

167 **Efficacy of spatial repellents varies depending on structure of the protected area**

Frances Golden, frances.golden@usda.gov, Seth Gibson, Daniel Kline, Robert Aldridge, Barbara Bayer, Adam Bowman, Maurice Solomon, Benjamin McMillan, Kenneth Linthicum, Nagarajan Ramasamy Rajagopal, Christopher Batich, Jeffrey Wahl

The efficacy of a spatial repellent may depend upon the structure of the protected area. For example, some spatial repellents may work best in enclosed spaces, while others perform better in open areas. We deployed the spatial repellent transfluthrin using a system of small polypropylene 3D-printed capsules containing cotton wool substrate in two different scenarios at Camp Blanding in Starke, FL, to determine how well each performed at reducing mosquito trap collections, and whether performance may vary by species. The first scenario was comprised of one-person US Marine Corps tents, with the capsules containing transfluthrin hung in the open doorway and a CO₂-baited CDC trap with light suspended inside from the middle of the tent roof. The second scenario consisted of a series of open-topped enclosures constructed of metal poles wrapped in US Army camouflage netting material on four sides, with the capsules containing transfluthrin strung across the top opening and a CO₂-baited CDC trap with light suspended in the center of the enclosure. In both scenarios, trapping for mosquitoes was conducted for one night approximately once a week, followed by identification and tallies of all collections to species, and calculation of percent reduction in collections for each scenario relative to collections in untreated controls. We found that transfluthrin substantially reduced collections in open-topped camouflage netting enclosures yet either had no effect or increased collections in the confines of small one-person tents. Moreover, we found that the efficacy of transfluthrin in reducing collections varied in some cases depending on the target species. These results provide evidence that implementation of spatial repellents should not be universal but rather should be balanced by knowledge of efficacy in the use scenario and against specific targets.

168 **Current Interest in Non-DEET Insect Repellents, Efficacy, Safety and the “Clean” Movement**

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Market studies show a growing trend towards natural and clean ingredients in cosmetics, personal care products and insect repellent products. Consumers desire safe, effective alternatives for insect repellents that are N,N-diethyl-meta-toluamide (DEET) free. Although DEET is safe and effective when used as directed, there is a perception that it may be harmful if used above recommended levels and many consumers prefer to use natural or nature-identical ingredients for their family. Ethyl butylacetylaminopropionate more commonly known as IR3535® is classified by the EPA as a biopesticide. Biopesticides are considered as a reduced risk pesticide, they are naturally occurring substances or synthetically derived equivalents that have a non-toxic mode of action to the target pest(s). Biopesticides produce little or no toxic residue, tend to decompose quickly resulting in lower exposures and minimizing pollution concerns typical of conventional pesticides. This presentation will review recent dermatological testing on volunteers with sensitive, atopic skin, mosquito repellency and

safety data that makes IR3535® suitable for use on adults and young children. IR3535® was also tested for repellency of lice, various species of flies, ticks, bees, and wasps with very favorable results. Cage and field test studies with *Aedes aegypti*, *Ae. albopictus*, *Anopheles* sp., *Culex* sp. show protection time between 8-12 h. IR3535® is shown to be nontoxic to aquatic organisms, non-persistent with no bioaccumulation in aquatic systems. Using sewage treatment simulation assay OECD 303A resulted in biodegradation at 99% with complete mineralization. The repellent is rapidly biodegradable in soils and not considered to be persistent or bioaccumulating. Studies show that IR3535® is rapidly biodegradable in sediment and not persistent or bioaccumulating in the sediment. Conclusion; IR3535® is well suited to meet the growing concerns for sustainable development goals for human health and well-being, responsible consumption and production, concern for life on land and in the waters.

169 **Spatial repellent efficacy in outdoor open enclosures in a north Florida forest**

Barbara Bayer, barbara.bayer@usda.gov, Robert Aldridge, Adam Bowman, Frances Golden, Daniel Kline, Maurice Solomon, Nagarajan Ramasamy Rajagopal, Christopher Batich, Jeffrey Wahl, Jeffrey Bloomquist, Seth Gibson, Kenneth Linthicum, , , ,

Treating the perimeter of US military materials, such as camouflage netting, with standard residual pesticides may protect the health and morale of US military personnel in the field by reducing incursion of disease vectoring or nuisance biting insects, such as mosquitoes or sand flies, into protected areas. However, standard residual pesticides rely on target insects resting on treated surfaces to accumulate lethal doses, while those that do not contact the treatment may still host seek. Furthermore, high mortality and sub-lethal exposure from standard residuals can result in the development of resistance and subsequent loss of efficacy. Emerging availability of diverse spatial repellents and toxicants, such as transfluthrin, offers an alternative to standard residual formulations and, if used to maximize their spatial repellent characteristics and not rely on mortality, are less likely to induce resistance. Residual applications of spatial repellents form a volatile plume, allowing target insects the opportunity to escape prior to lethal exposure and interaction with a host. While mortality from spatial repellents may occur if insects linger or become trapped in the volatile plume, those escaping will help maintain a population of susceptible insects. In this study we investigated the capability of transfluthrin and a novel formulation with the potential to be a spatial repellent, to repel endemic mosquito populations from entering protected camouflage netting enclosures and present differences in efficacy when deployed in a north Florida forest.

170 **EFFICACY OF DIFFERENT PLANTS SMOKE (EMANATOR) AGAINST Aedes MOSQUITOES**

Shabab Nasir, flourenceshabab@yahoo.com

Mosquitoes are known as vectors of many life threatening diseases like malaria, filariasis, Japanese encephalitis, Zika virus, chikungunya and dengue fever. In tropical countries over 2 million people are at risk of mosquito borne diseases. Among all, *Aedes aegypti* are becoming the most important vector of many virus such as dengue virus. The only solution to avoid these diseases is to manage the mosquito population. For this purpose, chemicals are used blindly. So, this method is a serious threat for human beings and environment because of insecticidal pollution. Hence, focus is being done on the use of plants. The repellent effects of the smoke from the leaves of selected plants; Neem (*Azadirachta indica*), Red gum (*Eucalyptus camaldulensis*) and Niazboo (*Ocimum basilicum*) was evaluated individually and in mixture against *Aedes aegypti* by direct burning and thermal expulsion

methods of application under normal lab conditions. Twenty five mosquitoes were taken in each treatment and let down effect data was taken three times for twenty minutes with 10 minutes break / halt between experimentation. The studies reflected that smoke posed negative effect on the adult behavior. Niazbo smoke showed more repellency and potential in causing maximum number of adult mosquitoes to let-down in case of thermal and direct exposure method. First time smoke exposure revealed maximum repellency in comparison with second and third. Niazbo also showed best results in combination with other plants. The minimum effective powder was Redgum, exhibiting minimum number of repellency against adults during first, second and third exposure time. The results also showed that direct exposure method was more lethal and effective against adult mosquitoes than thermal method.

9th Annual AMCA Arthropod Vector Highlights Symposium

171 Highlights of Vector Biology

Tara Thiemann, tthiemann@pacific.edu

There are over a dozen vector-borne pathogens responsible for human disease in the United States. These pathogens are transmitted primarily by mosquitoes, ticks, and fleas. It is crucial to understand the biology of these vectors, in order to comprehend, and therefore limit, pathogen transmission. This talk will review the latest findings in vector biology, based on peer-reviewed journal articles published in 2022. Articles will be found by searching bibliographic databases and internet sources, as well as by talking with experts in the field. The talk will focus on findings relevant to aspects biology that affect vector-borne pathogen transmission, as well as discoveries that are novel, unique, or awe-inspiring.

172 Highlights of control technology - 2022

Robert Aldridge, robert.aldridge@usda.gov

Following the historical tradition of "Highlights Symposia" in other professional associations, the published literature describing mosquito and vector control technologies in 2021-2022 was reviewed. From these works, a selection of publications was chosen to be "highlighted" by describing the authors' various methods and findings as they relate to mosquito and vector control technologies and how they can be applied to mosquito and vector control districts and organizations. Emphasis was placed on describing articles related to climate change, overcoming insecticide resistance, and disaster response including hurricane preparation and global pandemic guidance.

173 Highlights of European mosquito research

Alexandra Chaskopoulou, achaskopoulou@ars-ebcl.org

The increasing incidence of locally acquired mosquito-borne diseases in Europe over the past decade, combined with the introduction and growing geographic expansion of mosquito-borne pathogens have stirred the focus of the scientific community towards the development of new tools and approaches to better prepare for and contain this emerging threat across Europe. To that direction an upsurge of research has been observed towards improving our understanding on mosquito-pathogen-

host interactions under field conditions and designing improved surveillance and management practices. Furthermore, experts across academia, government, and industry, with diverse disciplinary backgrounds have joined forces and created collaborative support networks and research consortiums for producing new knowledge on vector-borne diseases (VBD) while also strengthening VBD preparedness and response. This presentation will focus on recent advancements (2021-2022) on mosquito biology research in Europe prioritizing topics relating to vector-pathogen ecology, vector-pathogen surveillance, and management strategies. A literature review will be performed by searching bibliographic databases, internet sources and the websites of relevant public health and vector control authorities. Information will be further assembled by contacting leadership of major European networks/research consortiums. While there is a plethora of high-quality research produced across Europe, to honour the spirit of the symposium, this presentation will primarily showcase unconventional, innovative approaches and bizarre findings.

Aerial Applications: Nuts and Bolts Discussion on Mosquito Control Operations Symposium I

174 The use of AgDisp for predicting aerial adulticiding drift and deposit

Mark Latham, manateemcd@aol.com

AgDisp is a "first principles" science-based model that predicts spray drift from application sites. It was developed by the USDA Forest Service and was designed to optimize agricultural spraying operations and has detailed algorithms for characterizing the release, dispersion and deposition over and downwind of the application area (quoted from the EPA website). The model, as "AgDrift", was validated by the Spray Drift Taskforce for low-level, large droplet agricultural spraying operations, with limits placed on the maximum altitude, computational time, downwind drift distance and minimum droplet size. However, AgDisp (with the AgDrift limitations relaxed) is now the model of choice for EPA's Risk Assessments of mosquito aerial adulticiding applications, which are applied at higher altitudes with smaller droplet sizes in a complex environment with many obstructions and vegetative filters. The "open field" assumptions modeled by EPA overestimate real world drift and deposit levels from aerial adulticiding.

This presentation will introduce the audience to AgDisp modeling and discuss some of these issues.

175 Emergency Response Activities for Mosquito Control after Hurricane Ian

Broox Boze, bboze@vdc.net

Hurricanes often cause severe flooding and increased mosquito populations that overwhelm local, state, tribal and territorial governments. FEMA's public assistance program is designed to aid response and recovery activities of public-serving entities which can include mosquito abatement when certain eligibility criteria have been met. While federal emergency declarations are intended to remove regulatory burdens and ensure a timely response over large areas, the National Incident Management System (NIMS), in conjunction with FEMA, is also responsible for ensuring safe and effective mosquito control measures. Part of the eligibility review for reimbursement of mosquito abatement activities includes consultation between FEMA and the CDC, in addition to consultation with

the US Fish and Wildlife Service or National Marine Fisheries. FEMA also coordinates with agricultural or natural resource departments to ensure economically important species are not affected by aerial applications of EPA-registered products. This presentation will highlight the importance of state and federal partnerships when large-scale mosquito control activities become necessary.

176 **Evaluating Aerial ULV Applications in Salt Lake City**

Greg White, greg@slcmad.org, Ary Faraji, Christopher Bibbs, Andrew Rivera

At Salt Lake City Mosquito Abatement District (SLCMAD) the vast majority of the mosquitoes arise from wetlands to the west of the city, next to the Great Salt Lake. During the summer months aerial ULV applications are the most effective means to control mosquitoes from this large habitat. SLCMAD evaluates how effective the aerial ULV applications are through monitoring mosquito populations, collecting droplets and using sentinel mosquitoes. In 2022 SLCMAD did an evaluation of the product Duet HD, which had not been used previously in the District. Some of the challenges to conducting aerial ULV applications in SLCMAD include low humidity, high elevations, erratic winds from being between the Rocky Mountains and the Great Salt Lake, an international airport adjacent to application areas and now a new state prison in the middle of the wetlands. Because of these environmental conditions and the restrictions from the airport we have our best control from dense products. The methods, equipment, and results of the evaluation conducted just west of the Rocky Mountains will be discussed in this presentation.

177 **Taking flight with a varied fleet: advantages of operations with a De Havilland DHC-6-100 “Twin Otter” and a Bell 407 GXi**

Kevin Caillouet, caillouet@stpmad.org, Nicholas DeLisi, Paul Spadoni, Josh Foulon, Calvin Smotherman

St. Tammany Parish, Louisiana covers nearly 900 sq. miles of coastal marsh, riverine swamp, and upland areas with heavy clay soils. Receiving more than 60 inches of rain on average each year and frequent tropical systems, Louisiana is no stranger to water and the mosquitoes excess rain and humidity bring. Traditional mosquito control methods in the state rely heavily on truck and large block airplane applications of adulticides. The 2017 acquisition of a De Havilland DHC-6-100 “Twin Otter”, replacing a King Air turbo lost in a 2016 crash, meets the large block need by safely treating up to 30,000 acres in one load. Determining aerial insecticide application efficacy against the primary West Nile virus vector, *Culex quinquefasciatus*, by measuring pre-to-post application abundances has been equivocal. In 2022, we monitored the efficacy of aerial Dibrom applications by comparing pre-to-post application in: 1) landing rates, 2) trapped abundances of *Cx. quinquefasciatus*, and 3) the parity rates of *Cx. quinquefasciatus*. Across 138 paired pre/post landing rates, mosquito abundance decreased significantly ($P < 0.001$) following aerial application from an average of 8.1 per minute pre- to 3.3 post-. Preliminary results from ongoing trials indicate no decrease in *Cx. quinquefasciatus* trap abundance after aerial treatment ($P = 0.995$). We measured a significant decrease in mosquito age ($P = 0.012$) from an average of 50% parous females pre- to 25% parous post-application. The 2022 acquisition of a Bell 407 GXi helicopter promises to give the St. Tammany fleet variability to perform both widescale and precise applications of adulticides while the flexibility to perform aerial larvicide missions in the years to come.

Barrier Treatment by Aerial Application for Control of Flooding Water Mosquitoes in Residential Areas

Rui-de Xue, xueamcd@gmail.com

Flooding water mosquitoes mainly caused nuisance problems and outbreak of West Nile and Eastern Encephalitis at sometimes. The populations of flooding water mosquitoes usually outbreak after 4-5 inches of rain fall in St. Johns County, northeastern Florida. During the development of residential communities /subdivisions, the trees and vegetation have been cut/removed and the lots have been filled and brought up. The retention ponds and ditches have been digging between the subdivisions and woody area. Most of the woody area become wetland or the area where is suitable for mosquito larval breeding and adult activity, such as sugar/plant feeding, water drinking, and hiding. Control of the mosquitoes around these areas become a challenge by ground application due to inaccessible problem. In the past few years, Anastasia Mosquito Control District, St. Augustine, Florida has adopted a new strategy to conduct barrier treatment of specific zone/edge between the residential subdivision and the woody area by aerial application. Based on positive larval survey and increased number of adult mosquitoes detected by human landing rate counts (LRC) and CDC light trap collection, an aerial larviciding mission with granule methoprene at 10 pounds/acre or an aerial adultciding mission with naled product at 0.6 O.Z. per acre were planned and conducted. For the larval control mission by aerial application, the helicopter flew one pass spraying on the edge between the woody area and subdivision. The spraying swap is/covers 300-400 ft. For aerial adultciding, the helicopter flew one pass spraying. The swap is and covers 1,500-3,000 ft based on the variation of wind speed. Based on the comparison of data (number of resident complaints or service requests, LRC, number of positive larvae survey and number of trap collection) pre-treatment and post-treatment, the barrier treatment at the edges between residential subdivision and woody area by aerial larviciding provided 80%-100% control of flooding water mosquitoes. The barrier treatment for the edges between residential subdivision and woody area by aerial adultciding provided 90%-100% of control of adult mosquitoes. The aerial barrier treatments are not only providing effective control of larval and adult mosquitoes, but also reduce the amount of pesticide applications and hours used for a whole area spraying. In addition, this strategy and method benefit to environments and have received more positive responses from the residents.

US Air Force Aerial Spray: The role of the DoD in Pest Management Activities

Deanna Scheff, deanna.scheff@usda.gov, Karl Haagsma, Mark Breidenbaugh, Jennifer Remmers

The US Air Force has a long history of aerial applications for the protection of US troops from arthropod-borne disease vectors dating back to World War II. Today, tactical command of the Air Force Aerial Spray Unit resides with the US Air Force Reserve and is assigned to the 910th Airlift Wing at Youngstown Air Reserve Station, Ohio. The Aerial Spray Unit (ASU) utilizes a modular aerial spray system (MASS) developed for use with C-130H airplanes. The Aerial Spray Unit conducts ongoing mosquito and herbicide control programs at multiple locations throughout the continental US. These spray missions allow entomologists, pilots, navigators, etc. to remain combat ready when and if they are called to deploy and protect our troops or civilians from arthropod-borne diseases in a variety of geographical locations. A supplemental but equally important role of the ASU is to mitigate effects of invasive species on DoD and federal properties. The ASU also responds to requests from the state and federal agencies to natural disasters such as hurricanes. The Aerial Spray Unit is tasked under DSCA (Defense Support to

Civilian Authority) to control mosquitos or other disease vectors that may threaten human health or to are at unacceptable nuisance levels for local recovery operations. The most recent activation of the Aerial Spray Unit was in Louisiana in response to Tropical Storm Cristobal and Hurricanes Marco, Laura, Delta and Zeta. Eighty-three airmen made deployed to Louisiana and successfully flew 60 hours, 11 sorties, and sprayed >900,000 acres over the most affected areas. The response by the USAF to the Hurricanes that affected Louisiana, will not be the last. The CONUS role of the Aerial Spray Unit is to support local vector control agencies when local agencies are incapacitated or overwhelmed and/or the use of contract applicators is unfeasible or unavailable.

Behavior/Biology I

180 Attraction of sand fly larvae *Phlebotomus papatasi* to larval feeding media and investigation of chemoattractants

Maia Tsikolia, m.tsikolia@gmail.com, Dimitris Tsaousoglou, Michail Miaoulis, Alexandra Chaskopoulou

Phlebotomine sand flies are important vectors of pathogens of medical importance, such as the *Leishmania* parasites responsible for 700,000 – 1 million new cases of leishmaniasis every year. For developing novel and targeted vector control tools it is important to improve our understanding on the ecology of sand flies across all different stages of their development. While there are numerous studies investigating attraction and oviposition preferences of adult sand flies, the ecology of larvae remains comparatively less known. The aim of this study was to explore the effect of different larval feeding media in attracting sand fly larvae. Two different bioassays were conducted a) a no-choice and b) a choice bioassay. No choice assays were used to determine the optimum time when 90% of the sand fly larvae reaches the standard feed mixture in comparison with the control (the pot with no feed). At the optimum time the majority of the larvae were found in the standard food. The choice assays were used to compare the feeding preferences between the standard feed medium, and other feeding substrates. Gas chromatography mass spectroscopy (GC-MS) equipped with the automated headspace (HS) system was applied to determine differences between the molecular compositions of the study materials, and by eliminating the identical components, identify molecule(s) that possibly produce the feeding attraction. These attractant molecules could be used to improve larviciding treatments by increasing contact of the larvae with insecticide, or/and as the lead structures for development of new active compounds.

181 A Closer Look: Field Wind Conditions Underlying Mosquito Responses

Neil Vickers, neil@slcmad.org, Agastya Balantrapu, Nick Conlin, Kirsten Meredith, Greg White, Ary Faraji, Marcus Hultmark

Turbulent conditions in the lower atmosphere are an inherent characteristic of the field environments through which female mosquitoes must follow passive scalar plumes in order to locate hosts for a blood meal. In recent field experiments conducted in different habitats, we have simultaneously measured prevailing wind conditions together with trap catches of carbon dioxide-responding female mosquitoes registered by a BG Sentinel trap equipped with a BG counter. In addition, we concurrently visualized flow conditions over larger scales by releasing either small bubbles or smoke.

We report on the correlations between different wind variables and the ability of female mosquitoes to respond to CO₂ plumes released by the trap. Using individual trap event time-stamps from the BG counter, we were able to focus in on specific time periods of mosquito activity to determine more precisely the wind condition variables that facilitate responses. Using insights from these experiments will permit us to replicate field turbulence within a large laboratory wind tunnel in order to study the behavioral mechanisms that these insects utilize in order to efficiently navigate toward potential hosts.

Funding support from the Pacific Southwest Regional Center of Excellence for Vector-Borne Diseases funded by the U.S. Centers for Disease Control and Prevention (Cooperative Agreement 1U01CK000516) (NV) and NSF-EAGER awards 2132726 (NJV) and 2132727 (MH).

182 **Bionomics and phenology of *Culiseta particeps* (Culicidae) mosquitoes in Lake County, California with considerations for changes in land-use and wildfire**

Cassandra Urquhart, october.sapphirina@gmail.com, Brittany Nelms, Tara Thiemann, Michelle Koschik, Jamesina Scott

Culiseta particeps is an uncommon and rarely studied mammalophilic mosquito species with a known range along the west coast of the United States and Canada, from Alaska to Mexico, with some collections made in Arizona. Little research has been done on the seasonality or overall bionomics of the species. The goal of this study was to address gaps in previous research and investigate aspects on the bionomics of *Cs. particeps* including host selection, parity, overwintering behavior, and larval and adult seasonality in Lake County, California. Adult collections were made to target both host-seeking and resting mosquitoes using traps, as well as vacuum aspirated collections from large red boxes and underground, man-made environments. Larval collections were made via dip sampling. All mosquitoes were identified to species, where possible. Determination of aestivation or overwintering status was evaluated through dissections of ovaries of empty (non blood-fed or gravid) adult females. A total of 52 blood-fed *Cs. particeps* were collected in Lake County and bloodmeals identified to determine host preference. Collection results and locations have been altered in recent years due to land-use changes and habitat altered by wildfires. This study will provide future investigators with additional knowledge of this species aiding in the broader understanding of mosquito biology and ecology and potentially the impact of natural disasters and human interference.

183 **Automated differentiation of mixed populations of free-flying mosquitoes**

Brian Johnson, brian.johnson@qimrberghofer.edu.au, Michael Weber, Martin Geier, Gregor Devine

Great advances in automated identification systems, or ‘smart traps’, that can remotely differentiate insect species have been made in recent years, yet convincing assessments of field-ready devices under free-flight conditions remain elusive. Here, we describe the results of the first mixed-species assessment of an optoacoustic smart trap design under free-flight conditions. Point-of-capture classification was attempted against congeneric (*Aedes albopictus* and *Aedes aegypti*) and non-congeneric (*Ae. aegypti* and *Anopheles stephensi*) container-inhabiting species of medical importance. The commonly co-collected *Culex quinquefasciatus*, a notable disease vector as well, was included as a third species in all assessments. Post-capture data cleaning, coupled with high species-level classification accuracy, resulted in a near perfect consensus between automated and physical trap counts across all

release scenarios. The discriminatory success of the tested system is credited to its unique design and sensor arrangement that allowed for a tenfold increase in signal length relative to commercially available alternatives. Increased signal length enabled accurate species differentiation despite significant overlaps in wing beat frequency distributions. The results presented strongly encourage the continued development of optoacoustic smart trap designs targeting mosquitoes of medical and economic importance.

184 **The biology of mermithid nematodes and their prospective use in the biological control of mosquitoes**

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Nematodes of the Mermithidae family are obligate endo-entomoparasitic of arthropods as insects. These nematodes are appealing for biocontrol due to their tendency to infect a large percentage of host populations. Mermithids have a simple life cycle consisting of 4 molts. Specifically, these nematodes hatch as infective juveniles (the only parasitic stage), who swims vigorously and hunt for an appropriate host and spend few days inside their host. The juveniles complete their development into postparasites while inside the host, and emerge from the host to dig into the floors of these mosquito pools changing to a free-living post-parasitic stage where they undergo the final two molts. The adult stages who is also a curious one, as the chance of finding a mate should be difficult in aquatic environment, mate, and lay eggs. For many years, mermithids have received attention for their usefulness as biological alternatives for the chemical insecticides, due to their potential killing efficacy, ease of rearing in mass quantities and their wide but specific host range. More importantly, these organisms are very safe to the environment and to non-target organisms, and no known resistance has developed against these parasites. *Romanomermis* and *Strelkovimermis* are mermithid nematodes that parasitize mosquito larvae. The behavioral and biological relationships of these mermithids with *Culex pipiens* were investigated. Particularly, differences in host penetration, emergence patterns, search behaviors for the host/mates, and population regulation are described. Results indicated that both nematodes, show different entrance and emergence sites. searching behavior has been shown to be directed, as they can detect the presence of their hosts and change their spatial distribution to attack them. Also, as parasite load increased, host heart rate and nematode survival decreased significantly, and both show discriminating against previously infected hosts. Species differences could be useful in developing production and release strategies.

185 **Attraction to host kairomones by male *Aedes aegypti* and *Aedes albopictus* mosquitoes in an air flow olfactometer.**

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Most mosquito reproduction starts with swarms of males waiting for a female candidate to present itself. In *Ae. aegypti* and *Ae. albopictus* these swarms can occur around potential blood meal hosts such as humans and in conjunction with positive phonotactic behaviors to female mosquito wing beat sound the male mosquitoes find female partners. The way males locate potential hosts or why however is little understood. In this experiment we have designed an airflow olfactometer to test close range responses of male mosquitos in both *Ae. aegypti* and *Ae. albopictus* to host kairomone responses. *Ae. aegypti* has shown positive responses to human odor and breath stimuli.

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Diel Activity Patterns of West Nile Virus Vectors in Fort Collins, Colorado

Dominic Rose, lvd9@cdc.gov, Broox Boze, Saul Lozano, Juan De Leon Rivera, John-Paul Mutebi

The aim of this investigation was to determine the diel activity patterns of all known West Nile virus (WNV) vectors to guide and improve the effectiveness of adulticide treatments. During June–October 2022, we conducted hourly collections over 96-hour periods each month using rotator traps to study host-seeking patterns of all WNV vectors. Preliminary observations indicate the host-seeking activity of *Culex tarsalis* Coquillett, *Cx. pipiens* Linnaeus, and *Cx. erythrothorax* Dyar is bimodal. Peak host-seeking activity occurred at dawn and during 9:00 PM–1:00 AM. These peaks shifted during September to the hours just after dawn and just before dusk likely because of changing photoperiod and daytime temperatures. These observations will be discussed in further detail.

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Mobile Insectaries: Mosquitos “On the Go”

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Mobile Insectaries: Mosquitos “On the Go” 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.

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A high-tech cinematic journey through the behavioral compendium of *Sabethes* mosquitoes

Robert Hancock, RHANCOC5@MSUDENVER.EDU, Nicola Zaragoza, Taylor Boyd, Shannon MacFadden, Connor O'Brien-Stoffa

In addition to their striking iridescence and ornamentation, mosquitoes in the Neotropical Genus *Sabethes* exhibit exquisite courtships, egg-throwing through tiny holes, wicked larval predation on other mosquitoes, nose biting, and more. Modern high-tech imaging has greatly increased our ability to record ethological details at high resolutions, high speeds, and high magnifications. We are using high-speed micro-videography with specialized optics to both describe new behaviors and to shed new light on others previously described. Our 10-minute narrated scientific film will include: 1) a brief introduction to Panamanian and path-finding “sabethologist” Pedro Galindo and a summary of his 20th century descriptions of behaviors exhibited by the forest yellow fever vector *Sabethes* (*Sabethoides*) *chloropterus* (Humbolt, 1819); 2) newly published videos of *Sabethes* (*Sabethes*) *cyaneus* (Robineau-Desvoidy, 1827) larvae using their siphons to snare larval prey; 3) possible sexual

signaling through male antennae and halteres movement that accompany the complex leg movements in the courtship of midleg-paddled *Sa. cyaneus*; 4) new work on the angular capabilities and accuracy of both aforementioned species in “egg-throwing” oviposition and how wing movement and appendage positioning change with egg-release angle; and 5) the curious act of nasophily by biting females.

The Results Are In: National, Regional, and Local Vector Control Capacity and Needs Symposium

189 Reactive Vector-Borne Disease Policy in the US Creates Opportunities for Improvement

Jennifer Gordon, jennifer@buglessions.com, Kyndall Dye-Braumuller, Kaci McCoy, Danielle Johnson, Rhoel David Ramos Dinglasan, Melissa Nolan

Vector-borne disease outbreaks repeatedly occur within the U.S. and surrounding territories. Dengue, eastern equine encephalitis, and West Nile outbreaks have occurred in recent years, and the next future outbreak is a matter of when, not if. Climate change, expanding urban environments, and insecticide resistance create favorable conditions for vectors to thrive and increase the chance they will come into contact with humans. To protect Americans, mosquito abatement programs manage mosquitoes and other vectors in the environment; however, these programs often rely on public funding to perform the necessary actions such as surveillance, management, and research. A recent investigation exploring federal grants awarded between 2008-2020 for vector control related efforts found that funding follows emerging/re-emerging vector-borne disease and decreases once the threat becomes endemic or subsides. This talk will discuss the implications from the boom-bust cycle of reactive federal vector control policy, progress made in recent years to correct this trend, and what actions policy makers and constituents can make to address the current national inadequacies in vector-borne disease policy and infrastructure.

190 Vector Surveillance and Control Capacity at the Local Level

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Objective: This session summarizes the findings from the 2020 Vector Assessment, conducted by the National Association of County and City Health Officials (NACCHO) in coordination with the Centers for Disease Control and Prevention (CDC). The results from the 2020 assessment are compared to the results from the 2017 assessment to track trends in mosquito surveillance and control capacity at the local level over time.

Methods: This is the second iteration of NACCHO’s nation-wide assessment of local vector control programs. The survey instrument was programmed in Qualtrics and sent to a database of 1,664 verified local vector control programs, which includes local health departments as well as mosquito control districts and other vector programs that may be housed in different branches of local or tribal governments. The response rate was 30 percent. The survey was fielded between November 2020 and January 2021 and the concurrent spike in the COVID-19 pandemic at that time is likely to have lowered

the overall response rate given the added strain it placed on local public health departments, which comprised a majority of respondents. Analysis of the results was completed in Stata.

Results: Most programs reported capacity for routine surveillance (68%), adulticiding or larviciding (98%), and routine vector control (79%). However, a majority of programs (72%) were still considered “needs improvement” given the lack of capacity for pesticide resistance testing. Encouragingly, between 2017 and 2020, mosquito surveillance and control capacity improved across most of the ten measures assessed, including nearly double the proportion of programs reporting capacity for pesticide resistance testing in 2020 as compared to 2017.

Conclusions: Mosquito surveillance and control capacity at the local level has improved between 2017 and 2020. Significant gaps remain with regard to pesticide resistance testing. In addition, there remain opportunities for improvement with regard to making evidence-based treatment decisions.

191 **Southeastern U.S. Vector Control Needs Assessment**

Kyndall Dye-Braumuller, kyndallb@email.sc.edu, Jennifer Gordon, Danielle Johnson, Josephine Morrissey, Kaci McCoy, Rhoel David Ramos Dinglasan, Melissa Nolan

A national 2017 vector control capacity survey was conducted to assess the United States’ (U.S.’s) ability to prevent emerging vector-borne disease. Since that survey, the southeastern U.S. has experienced continued autochthonous exotic vector-borne disease transmission and establishment of invasive vector species. To understand the current gaps in control programs and establish a baseline to evaluate future vector control efforts for this vulnerable region, a focused needs assessment survey was conducted in early 2020. The southeastern U.S. region was targeted, as this region has a high probability of novel vector-borne disease introduction. Paper copies delivered in handwritten envelopes and electronic copies of the survey were delivered to 386 unique contacts, and 150 returned surveys were received, corresponding to a 39% response rate. Overall, the survey found vector control programs serving areas with over 100,000 residents and those affiliated with public health departments had more core capabilities compared to smaller programs and those not affiliated with public health departments. Furthermore, the majority of vector control programs in this region do not routinely monitor for pesticide resistance. Taken as a whole, these results suggest that the majority of the southeastern U.S. is vulnerable to vector-borne disease outbreaks. Results from this survey raise attention to the critical need of providing increased resources to bring all vector control programs to a competent level, ensuring that public health is protected from the threat of vector-borne disease. Results can be compared to state-wide and national results of similar surveys to understand where gaps in mosquito-borne disease preparedness lie.

192 **Results from a Research Survey: Capabilities and Capacities for Vector Control in Florida and Texas**

Steve Peper, speper@amcdfl.org

A previously published study reported that about 80% of vector control organizations throughout the southern USA lack critical capabilities to address vector populations and vector-borne

diseases within their jurisdictions. This study also reported that only 26% of jurisdictions in Florida and 14% in Texas were considered “fully capable”. Both states are among the top 4 states relative to the number of human cases of mosquito-borne diseases and had local transmission of Zika virus in 2016. This study further investigated current vector control capabilities and capacity within the states of Florida and Texas. Florida jurisdictions indicated that 88% have vector control capabilities to some degree, with 65% of those reporting they had sufficient capabilities. Texas jurisdictions indicated that 89% have vector control capabilities to some degree, with 67% of those reporting they had sufficient capabilities. As the prioritization of resource commitment for vector control capabilities varies throughout the USA, it is imperative that each state evaluates their specific needs, current capabilities, and capacity to best ensure the public health needs within their jurisdictions through vector and mosquito control.

193 **Survey of United States Mosquito Control Programs Reveals Opportunities to Improve the Operational Value of Centers for Disease Control and Prevention Bottle Bioassays**

Stephanie Richards, richardss@ecu.edu, Brian Byrd, Mark Breidenbaugh, Kurt Vandock

Mosquito control programs have increasingly used Centers for Disease Control and Prevention bottle bioassays (BB) to evaluate insecticide resistance (IR). The reported utility, benefits, and limitations of BB and other methods were assessed via electronic survey of United States mosquito control professionals to identify potential areas for improvement, future study, and professional training. Surveys were administered to 249 mosquito control professionals across the US with a resulting 29% (N=71) response rate. Most respondents (94%) indicated that bottle bioassays are useful/critical to their mosquito control programs (MCPs) and this should be considered when interpreting results. Opportunities were identified to improve BB operational value and IR detection. Clear universal guidelines should be developed for the practical use of bottle bioassay data as it relates to operational impacts in MCPs. Financial support or other assistance (e.g., regional collaboration with mosquito associations, industries, universities) may be needed for long-term IR testing and/or field trials.

UAS in Mosquito Control Symposium

195 **Methods and applications for mosquito larval habitat identification and automated treatments**

Joseph Civello, jcivello@frontierprecision.com

Given the rising costs of equipment, fuel, treatment products, and shortages in staff combined with the advancements in UAS technology; there are many benefits to employing a data-driven method to identify targeted treatment areas and automating aerial treatments with Unmanned Aerial Systems (UAS). Unmanned Aerial Vehicle (UAV) mounted LiDAR and Multispectral sensors have become lighter and more cost-effective than ever before, allowing for widespread adoption and integration with off-the-shelf UAS platforms. Data from these sensors can help to model larval habitats, resulting in less time spent in the field, and fewer products being wasted on ineffective treatments resulting in overall efficiency and efficacy. This approach lends itself to automation with treatment drones, equipped with Real Time Kinematic (RTK) GPS and obstacle avoidance.

Reporting aerial treatments, proposed treatment areas, and UAV collected data with associated outputs is streamlined with the use of FieldSeeker GIS Software. Implementation of these methods will optimize workflows and reduce operating costs for your agency.

We will overview recent product releases, specifications & limitations, software updates, and general workflow suggestions.

196 **The role of drones in Collier Mosquito Control District's *Mansonia* control program**

Rebecca Heinig, rheinig@cmcd.org, Peter Brake, Andrew Weiss, Rachel Bales, Keira Lucas

In spring of 2022, Collier Mosquito Control District initiated a new surveillance and control program tailored to local *Mansonia* mosquito species. The pilot site for this program was a rural area with numerous irrigation ditches that provided abundant habitat for larval *Mansonia*, which are obligately associated with aquatic weeds such as water lettuce and water hyacinth. However, the program faced a number of challenges related to site access, habitat assessment, and navigation of the rapidly changing landscape. To meet these challenges, we turned to drones. During the field season, drones were used to deploy and retrieve traps, perform targeted larvicide applications, and generate up-to-date aerial images for use in site evaluation and treatment decision-making. This presentation will discuss the process of program development, preliminary efficacy assessments, and predicted impacts of new Florida legislation on the future of the program.

197 **UAS License and CEU requirements for VCD's**

Steve Shepherd, sshepherd@ocvector.org

We will describe the Federal and state requirements for becoming UAS operators in the vector control industry. We will also compare the different state licensing regulations and continuing education requirements for retaining licensure. Besides a useful guideline, this may also be the basis to drive consistency across various state agencies who regulate UAS operations and CEU requirements. The goal is to streamline VCD's administrative compliance to safely and efficiently operate UAS vehicles for the protection of public health

198 **Upsizing The Fleet – Utilization of the DJI Agras T30 and MG-1S for Mosquito Control Applications in Salt Lake City, Utah**

Bradley Sorensen, Brad@slcmad.org, Michelle Christian, Matthew Marcusen

The Salt Lake City Mosquito Abatement District has been using Unmanned Aerial Systems (UAS) to apply larval control products since 2018. In 2022, The District purchased a DJI Agras T30 to increase their ability to aerially apply mosquito control products. This new aircraft is in addition to an older DJI Agras MG-1S that is still operating within the fleet. Together the two aircraft worked side-by-side to treat around 2,000 acres of larval habitats during the 2022 season.

In this presentation we will talk about the growth of the UAS program in Salt Lake City and we will cover some of the challenges and issues encountered during the year. We will talk about the differences between the two models of aircraft used this year, including the types of support equipment needed to operate them. Lastly, we will talk about the different requirements and regulations involved in operating

an unmanned aerial system that weighs more than the Federal Aviation Administration's 55-pound weight restriction.

199 **Larvicide Applications with Unmanned Aircraft Systems in Placer County, CA**

Scott Schon, scotts@placermosquito.org, Ev Ortiz, Joel Beuttner

The Placer Mosquito and Vector Control District (PMVCD) has been applying larvicides via unmanned aircraft systems (UAS) since 2019. We started by conducting trials to provide baseline data for safe and effective larvicide applications to incorporate this technology into our day-to-day operations. We have treated over 880 acres; in sources including: organic rice fields, large retention ponds, snowmelt pools, irrigated pastures, and wetlands. The DJI AGRAS MG-1S can effectively treat mosquito sources that historically were treated with All Terrain Vehicles, or smaller hard-to-access sources that were treated by technicians using hand-held equipment. UAS provides us another effective tool in our toolbox, and we look forward to evaluating new, larger UAS in the future.

200 **Hylío - Precision UAS for Vector Control**

Arthur Erickson, arthurerickson@hyl.io

Hylío, Inc. is an American owned and operated company based in Richmond, Texas. Hylío designs, manufactures, and offers UAS to operators in a wide variety of industries including vector control, vegetation management, and agriculture. In his presentation, CEO and Co-Founder, Arthur Erickson, discusses the advantages and logistics of deploying precision UAS technology for mosquito control applications.

Advantages include, but are not limited to:

- Easily access treatment sites that are otherwise difficult to traverse with traditional vehicles (e.g. lakes, retention ponds, levees, marshland, etc.)
- Precisely and consistently apply products thanks to RTK GPS, digitalized material tracking, and automated controls
- Reduce human error by leveraging defined, repeatable autonomous treatment missions
- Mitigate risk product drift thanks to the UAS' propeller downwash and close proximity to the intended target zones
- Safer operations; avoid operators having to traverse potentially dangerous areas

Arthur also outlines general operational procedures and describes what to expect when using UAS for mosquito control.

201 **The PrecisionVision 35X UAS larvaciding and adultciding for an integrated mosquito management approach**

Bill Reynolds, breynolds@leateam.com

Over the last five-years, the PrecisionVision UAS have performed more than 6 independent ULV adultciding research projects and numerous operational applications. This

presentation will focus on the technologies that are required to successfully deploy UAS for aerial adulticiding and larvaciding applications.

Data from several field trials performed by independent mosquito control agencies and researchers will be presented. In September of 2022, an operational application that consisted of larvaciding and adulticiding in the Southern San Joaquin Valley will be discussed. Details specific to pre and post adult traps and larval inspections counts will be presented along with data sets associated with WNV transmission rates. Additional topics such as local resistance will be presented.

Conventional ground based application methods of larvicides and adulticides are often ineffective in reducing the mosquito populations to acceptable levels due to the dense canopy or limited access from roads. Unmanned aerial systems (UAS) have the potential to assist in controlling the mosquito populations in these challenging locations.

Aerial Applications: Nuts and Bolts Discussion on Mosquito Control Operations Symposium II

202 Aerial ULV in the Desert - Examining Efficacy

Kim Hung, khung@cvmosquito.org, Jacob Tarango, Marc Kensington, Arturo Gutierrez, Melissa Snelling, Jennifer Henke

Coachella Valley Mosquito and Vector Control District (the District) conducts aerial adulticide applications in response to arbovirus detections and to extraordinary adult collections. Applications began in 2012 when comparing results of truck and helicopter applications in the eastern valley, where virus had been detected annually. Results at that time indicated a more complete coverage of the targeted area, leading to improved reductions in mosquito populations and improved outcomes for residents living in the area. Aerial ULV applications have still been used typically in the eastern valley because the western valley has numerous golf courses with water features that include fish. In 2022, we made several applications of adulticide using ULV applications. Through this work, we had differences in our results from our expectations. This led to opportunities to compare applicators targeting the same area with similar techniques and different pesticides. We had some errors in our set-ups that we have learned to correct, as well as plans to improve our future applications.

203 Characterization of the spray distribution of Unmanned Aerial Spray Systems (UASS) and the development of turnkey systems for vector control

Jane Bonds, jasbonds@gmail.com, Brad Fritz, Harold Thistle

Systems for both adulticiding and larvaciding have been developed for the Deployed Warfighter Protection Program. This is the final report on the characterization of these systems in terms of the physical characterization and efficacy. The research has highlighted potential issues and provided solutions in terms of both engineering controls and operational protocols to optimize the systems.

204 An Overview of the Aerial Adulticide and Aerial Larvicide Program of Butte County Mosquito and Vector Control District

Ryan Rothenwander, rrothenwander@buttemosquito.com

An Overview of the Aerial Adulticide and Aerial Larvicide Program of Butte County Mosquito and Vector Control District

Butte County has a mix of land uses ranging from rice/orchard/pasture agricultural, wetland wildlife conservation, waterfowl recreational sport hunting, residential, and light industrial/manufacturing. Large swaths of the county are flooded throughout the year to facilitate such activities and, in doing so, create mosquito breeding habitat, which is a challenge to manage effectively from the ground or along the edges of large, flooded tracts of land.

To mitigate public health threats from 50,000 acres of managed wetlands and an average of 100,000 acres of rice effectively, Butte County Mosquito and Vector Control utilizes 3 aircraft with regular routines for spraying larvicides and adulticides over the District's service area. Areas are scouted and mapped on the ground by technicians and these maps are uploaded into Mapvision and SatLoc G4 used by the pilot to apply these pesticides.

As the season progresses and the rice and wetlands become overgrown, larvicide applications become ineffective due to the inability of the product to reach the target water source. As a final control measure, our aerial adulticide program will take over by applying an adulticide over an area with a high density of pestiferous mosquito vectors. In the event of a public health emergency, plans for an application over a densely populated town or city using a two engine aircraft are also available, yet rarely used.

This presentation will overview the aircraft, pilot, loader trucks, mapping systems, drift modeling technology, pesticides and maintenance of equipment used within BCMVCD's aerial larvicide and adulticide program.

205 **Shared aerial operations a unique endeavor**

Herff Jones, hjones@iberiagov.net

It is no surprise the southeastern United States is most vulnerable to the impacts of hurricanes, and tropical storms; particularly when these tropical weather events enter or generate in the Gulf of Mexico. Iberia parish is surrounded by several hundred thousand agriculture, aquaculture acres, bayous, streams as well as the nation's largest hardwood swamp. Iberia's southern border is the Gulf of Mexico and fully exposed to storm surge flooding from extreme tropical weather events. Typically, a named storm event will result in more than half of the state's parishes declared for emergency disaster relief. Conditions post-landfall quickly elicit enormous floodwater mosquito populations. Aerial mosquito abatement capability is an effective and particularly useful operational tool in these circumstances. Additionally, mosquito control by air is of great value to the citizens of our parish as they attempt to recover. The utilization of CDC grant funding to acquire, outfit, and modify an aircraft for aerial mosquito spraying will dramatically reduce the costs of providing this type of operational control and save valuable local tax dollars. Our in-house aerial operations will realize increased efficiency, efficacy and reliability without the need for complete reliance on contract services. More importantly, these enhanced operational capabilities will directly translate to added health protection for the citizens of Iberia parish. I hope to present the need for this type of operational control tool, the background related to the CDC grant, the unique cooperative endeavor with our neighbor

Vermilion Parish, the general details of the project and the current status of the project after our first full season of joint aerial operations.

206 **Evolution of the aerial program at Collier Mosquito Control District**

Rebecca Heinig, rheinig@cmcd.org, Keira Lucas, Patrick Linn

Collier Mosquito's aerial control program began in 1965 with a single Twin Beech. Today, we have a fleet of six aircraft that includes both fixed-wing and rotary units. Here, we'll discuss how our program has changed over the years as we've shifted from an adulticide-based approach to a more integrated management strategy and discuss the challenges we've faced as we've begun to update our older aircraft.

207 **From mission planning to efficacy assessment, a review of Sacramento-Yolo Mosquito and Vector Control District's aerial ultra-low volume adult mosquito control program**

Steven Ramos, sramos@fightthebite.net, Sarah Wheeler, Deborah Dritz, Mario Novelo Canto

The Sacramento-Yolo Mosquito and Vector Control District (District) has a long history of responding to West Nile virus activity and high adult mosquito abundance through the use of ultra-low volume (ULV) treatments using fixed-wing aircraft. These public health applications are conducted over a diverse range of geographic locations including agricultural settings such as conventional and organic rice, wetlands, and densely populated urban areas. Several factors drive whether Sac-Yolo MVCD conducts an aerial ULV spray mission including: mosquito abundance, detection of West Nile virus activity, proximity to population centers, and weather. The selection of adulticides is based on the season, area where they will be applied, and proven efficacy. Here, we will present and discuss all aspects of the Sac-Yolo MVCD aerial ULV program, starting with the factors that trigger an aerial ULV mission in both urban and rural areas, methods used to build spray blocks and application mechanics, and lastly, an evaluation of aerial ULV application efficacy and resistance management.

208 **Ultra Low Volume Naled Applications: Modeling and Literature Review Human Health Findings**

Daniel Mendoza, daniel.l.mendoza@gmail.com

Ultra low volume (ULV) naled applications for mosquito adulticiding are a widely used control method. Naled has been registered and used for over 60 years and regulatory review processes are conducted by the United States Environmental Protection Agency (USEPA) every 15 years. In this presentation we will review findings of a modeling study using two USEPA models – AERMOD and AgDISP quantifying both deposited and suspended (at 1m breathing height) naled concentrations following a typical ULV application using a range of meteorological parameters. The modeling study showed that potential naled exposure is well below the USEPA's Acceptable Daily Intake (ADI) even in the most extreme cases. Additionally, the results of a scoping review of published literature linking naled and dichlorvos (DDVP) – a metabolite of naled – and potential human health impacts will be discussed. The review found no evidence that ULV naled applications for mosquito control resulted in increased levels of naled in humans, provided the naled is used according to label instructions. Our findings are in congruence with EPA and CDC recommendations that aerial applications of naled, when applied

according to label requirements, do not pose an adverse risk exposure to humans, wildlife, and the environment.

209 Integrating sUAVs into Vector Control in order to increase efficiency and effectiveness, while remaining financially responsible

Edward Horvath, ehorvath@trmvc.com, Dongbin "Don" Lee, Danta Smith

Now, more than ever, mosquito control professionals are looking for ways to be more efficient and effective. Fuel prices have driven up the costs of conducting operations; from pesticides to vehicles and aircraft operations. UAVs, or drones are tools that vector control operations are utilizing to be more precise and efficient. sUAVs are being used for mosquito source surveillance, larviciding and ULV adulticiding. This collaboration between a university engineering department and Three Rivers Mosquito and Vector Control is developing sUAVs that are effective, efficient and affordable.

Aquatic plant-associated mosquitoes Symposium

210 Evaluation of larval control strategies targeting *Coquillettidia perturbans* in Pasco County, Florida

Agne Prasauskas, aprasauskas@pascomosquito.org

Coquillettidia perturbans is a wide-ranging, prominent nuisance mosquito, as well as a known bridge vector for eastern equine encephalitis virus and West Nile virus. They are strong flyers and vicious biters. Larval control of *Cq. perturbans* has always proved challenging due to its unusual habit of attaching to emergent vegetation and subsequent difficulty with its surveillance. However, another habit of the species, overwintering and the highly predictable emergence in the spring, should be taken advantage of for economical control. A study on the targeted spring larval control efforts of *Cq. perturbans* was undertaken in Pasco County, FL. Eight local retention ponds with confirmed breeding of *Cq. perturbans* were monitored for emergence from March through November of 2020 following treatment in late March. Two emergence traps were placed over clusters of matted floating cattails within each of the sites and collected weekly. Compiled emergence data was used to compare the efficacy of different control strategies targeting *Cq. perturbans* immatures.

211 Lee County Hyacinth Control District – 62 Years of Aquatic Vegetation Management

Colin Lewis, Lewis@lchcd.org

The Lee County Hyacinth Control District (LCHCD) manages aquatic vegetation on all public waterbodies in Lee county Florida, including the Caloosahatchee River and its tributaries. Aquatic vegetation management serves a critical role in southwest Florida by maintaining navigation channels for recreational access, ensuring proper drainage at flood control structures, and reducing populations of invasive vegetation which thrive in Florida's subtropical environment and can serve as breeding sites for mosquitoes. Recently, LCHCD has joined efforts with the Lee County Mosquito Control District (LCMCD) to target mosquito breeding hot spots throughout the county utilizing the latest control technologies. Unmanned Aircraft Systems (UAS), otherwise known as drones, allows LCHCD to target mosquito-breeding aquatic vegetation such as water lettuce, cattail, and water hyacinth in areas that

have been historically difficult to treat or inaccessible by standard conventional means. As technology improves, drones have the potential to revolutionize the way in which we access, target, and treat aquatic vegetation and mosquito populations.

212 **From Project to Program: Collier Mosquito Control District's Development of an Emergence Trap- based Surveillance Program for *Mansonia***

Rachel Bales, rbales@cmcd.org, Peter Brake, Andrew Weiss, Jesse DeBella, Keira Lucas, Rebecca Heinig

Larvae of the mosquito species *Mansonia titillans* and *Mansonia dyari* are unusual because they attach to the roots of floating aquatic plants, primarily water lettuce (*Pistia stratiotes* L.) and water hyacinth (*Eichhornia crassipes*), to obtain oxygen and avoid predation. Because the larvae do not move freely within the water column, conventional larval dipping practices are inefficient and misleading. For this reason, Collier Mosquito Control District's *Mansonia* surveillance strategy historically consisted of habitat identification and CDC light trap data. While this approach effectively demonstrated whether *Mansonia* spp. were in a general area, it lacked the level of precision that could be achieved using emergence traps, which reveal exactly where larvae are located and how many potential adults are emerging from a specific area of larval habitat. For this reason, the District has spent the last two years developing two robust emergence trap designs, demonstrating their functionality, and estimating population baselines. In 2022, the project moved into its operational phase. Emergence traps were deployed weekly throughout the field season in a pilot area. The data obtained was used to inform treatment decisions as well as evaluate treatment efficacy. Trap operational utility, larvicide performance, and plans for the upcoming year will be discussed.

213 **The challenges of *Cq. perturbans* an important Eastern Equine Encephalitis vector in Massachusetts**

Priscilla Matton, brismosqpc@comcast.net, Todd Duval, Ellen Bidlack, Kaitlyn O'Donnell, Frank Cornine III

Coquillettidia perturbans is one of the most common mosquitoes collected in Massachusetts. The mosquito is an aggressive mammal biter and is implicated in the transmission of Eastern Equine Encephalitis Virus (EEEV) to humans. The larvae are notoriously difficult to control or to research because they attach themselves to emergent vegetation with a modified siphon, which allows them to extract oxygen from root tissue. Following unprecedented EEEV activity during the 2019 season across the state, aerial applications to control the larval stage of this mosquito were planned. Targeted applications to *Cq. perturbans* producing wetlands using Vectolex® FG (*Bacillus sphaericus*) and Natular® G (spinosad) were conducted. Applications occurred in both the spring and/or fall using either fixed-wing aircraft or helicopter. Different surveillance methods were employed both pre- and post-application to determine efficacy of the treatment. Evaluation of efficacy was determined by larval counts and/or adult emergence during the current season or the following year.

214 **Overview of the Metropolitan Mosquito Control District's *Coquillettidia perturbans* control program.**

Mark Smith, mmcd_mes@mmcd.org

The Metropolitan Mosquito Control District developed a *Coquillettidia perturbans* control program over 30 years ago. This species has been a major contributor to the annoyance of the three million citizens of the Minneapolis & St Paul metropolitan area. This univoltine species utilizes permanent water habitat and physically attaches to established water roots of aquatic plants to obtain oxygen to breathe. The preferred host plant in our region is the common cattail (*Typha latiflora*) but other plants, such as sedges or grasses, can be utilized. Their preferred habitat requires long standing water in which the plant's water roots can fully develop over time. This species can be difficult to locate but District staff have found many other factors in which contribute to their successful surveillance of this species. This presentation will provide an overview of our cattail program and how it has evolved to control this possible vector species in our seven county region.

215 **Mansonia species source reduction: The search for a habitat, host plant, and herbicide**

Shannon O'Meara, someara@stpmad.org, Nicholas DeLisi, Sydney Johnson, Josh Foulon, Kevin Caillouet

St. Tammany Parish, Louisiana is rife with *Mansonia tittillans* breeding grounds, from 40 linear miles of coastal marsh on Lake Pontchartrain all the way down to neighborhood ponds and roadside ditches. These mosquito populations peak every fall and become the primary nuisance mosquito across the parish through the end of the year. Surveillance indicates *Mn. tittillans* rely on the invasive aquatic weed water hyacinth (*Eichhornia crassipes*) as their primary host plant in the region. Since fall of 2019, 57% of all emergence traps set upon water hyacinth in peak months have collected *Mn. tittillans*, while none have been found on water lettuce or giant salvinia. Conventional larvicides largely failed to control *Mn. tittillans* larvae in long-term field trials. As a result, St. Tammany Parish Mosquito Abatement has recently begun managing this host plant with herbicides applied by unmanned aerial vehicles and ATV-mounted sprayers. All herbicides tested in semi-field mesocosms effectively controlled water hyacinth, but the rate at which this occurred varied greatly. We have begun to evaluate non-target effects of herbicide application as one means of addressing public perception of herbicide use. While our program is still nascent, we hope to measure the mosquito abatement repercussions of source-removal of an invasive host plant over the coming years.

Behavior/Biology II/Adult Control I

216 **Improved Malaise Trap Systems for Sampling Mosquitoes**

Gunter Muller, guntermuller@hotmail.com, Rui-de Xue

Although Malaise-Traps are highly effective and unbiased in catching mosquitoes they are rarely used in surveys or experimental studies. To increase catches, we suggest several new practical modifications of Malaise-Traps.

Trials were conducted in urban and rural settings in Mali, West Africa, the main targets were mosquitoes of the *Anopheles gambiae* complex and *Culex* species.

Observations with night-vision equipment showed that mosquitoes are often readily entering the net constructions but they seldom get caught in the passive collecting containers.

Catches were significantly increased when the regular containers were replaced by battery powered suction devices (as used in CDC-Traps). Best results were obtained if the suction device was combined inside a 40cm long dark plastic tube with LED UV light diodes. The UV-light which was visible in the tube, was directed to throw light only in a perimeter of 20cm of the net and thus it affected only mosquitoes which were already inside the net. Anopheline catches increased up to 15 X and Culex catches up to 11 X with different types of Malaise-Traps and suction heads. The modified Malaise traps outperformed by far the conventional CDC-UV traps, both in rural and especially urban settings. Additionally, Malaise traps caught significantly more blood fed females which are often scarce or absent in UV-CDC traps.

217 How to assess the attraction distance of mosquitoes to blood and sugar feeding sources

Gunter Muller, guntercmuller@hotmail.com, Rui-de Xue

Experiments were carried out in the flood plain of the Niger in Mali West Africa, in rice field areas in which Culex sp. and Anophelines of the gambiae-complex were breeding in large numbers. A trapping pattern of two concentric Malaise trap circles, separated by a distance of 300m from each other, with radii of 6m up to 50m, was employed. One circle was alternately used as control and the other as experimental. Cows, humans, chickens, and different types of flowering and non-flowering vegetation were used as attractants in the center of one circle while the control circle was without a bait. For each experiment two identical circles of Malay traps, with radii that were presumed suitable for the tested attractant were set up. According to the results, the following nights, traps were positioned in shorter or longer distances from the same bait. The caught mosquito females in the two circles were identified and counted. A statistical difference (after ten repetitions) between control and experimental catches was considered attraction. Culex and Anophelines were attracted differently. Maximal attraction distance varied significantly from bait to bait, varying from no attraction at 4m with attraction up to 48m.

218 The Effect of sublethal doses of Glyphosate on the Development and Fitness of Culex quinquefasciatus

Mahmood Nikbakhtzadeh, nik.nikbakht@gmail.com

Glyphosate is the world's most widely used herbicide. Most of the herbicides applied in agriculture do not reach their target sites and are therefore found in considerable levels beyond agricultural fields. Glyphosate similarly leaves a target site through surface runoff, where it has been detected in aquatic systems frequently. Culex quinquefasciatus Say, more commonly known as the southern house mosquito, is one of the most widespread mosquitoes in southern California. This species is responsible for transmitting arboviruses such as West Nile virus (WNV) and St. Louis encephalitis (SLE) to the local residents.

In our previous studies, we have already tested the effect of higher doses of glyphosate on the development and survival of Cx. quinquefasciatus larvae and also investigated the effect of high doses on the female oviposition. In our current study, we will investigate the effect of sublethal doses of a commonly-used formulation of glyphosate on the oviposition, larval development and adult fitness of Cx. quinquefasciatus under laboratory conditions.

Roundup® Weed and Grass Killer, Super Concentrate, will be used as a commercially available glyphosate product (50.2% glyphosate isopropylamine salt; Monsanto). Serial concentrations (1000 µg/l, 100 µg/l, 10 µg/l and 1 µg/l) of glyphosate will be made from concentrate and tested. Glyphosate formulations (mixed with adjuvants) have been reported to be more toxic than pure glyphosate, and these are the formulations available on the market which are also used in agricultural operations. This research adds to our understanding of the factors which impact the vectorial capacity, survival and behavior of *Cx. quinquefasciatus*. These are important features of a vector which spreads human diseases.

219 **Projecting spatial and ecological predictors of arbovirus transmission risk**

Joseph McMillan, josmcmil@ttu.edu, Luis Chaves, Philip Armstrong

Using mosquito and arbovirus data from the Connecticut Agricultural Experiment Station's (CAES) statewide mosquito and arbovirus surveillance program, we developed quantitative methods that project arbovirus risk at the scale of surveillance sites within a network as well as in un-sampled spaces that surround sites within a network. For among site projections, we utilized population synchrony methods which defined spatiotemporal relationships of mosquito and arbovirus collections among sites as well as investigated the climatic and environmental variables associated with high synchrony estimates. For risk projections into un-sampled space, we used machine learning methodologies to first develop predictive algorithms of *Culex pipiens* or *Culiseta melanura* collections based on climate, land cover, and prior monthly collections; these algorithms were then nested within a predictive model of West Nile virus or eastern equine encephalitis virus detection probabilities. The over-arching goal of this research is to develop interactive, online risk maps which can be released to the public by CAES during a surveillance season. The predicted utility of such risk maps is that they will allow users to estimate arbovirus risk at sites within the surveillance network which may test negative for a virus or in locations not explicitly sampled by the surveillance network.

220 **Modifying the Standard Topical Bioassay: an Innovative Method for New Modes of Action**

Joanna Tyszkowski, jtyszkowski@clarke.com

Modifying the Standard Topical Bioassay: an Innovative Method for New Modes of Action Topical dose response applications are an essential step in the process of assessing potential active ingredients for viability in mosquito control. The standard topical application methodology, utilizing Burkard and Hamilton micro-applicators, has long been used for topical bioassays and applies a known dose of insecticide directly to the exoskeleton of insects, thereby ensuring exposure to a specific amount of compound. Development work to identify and design next-generation mosquito control products featuring new active ingredients, new modes of action, and new formulation science has laid the groundwork for innovation in dose-response methodologies. This presentation will explore a novel topical dose-response method developed and evaluated in Clarke's bioassay lab using Drummond Nanoject. With this method and equipment, nanoliter-sized droplets of prototype formulations, diluted with formulation inerts, can be applied onto the mosquito cuticle, allowing droplet behavior and effect on the mosquito to be observed in new ways compared to the traditional methods. This innovation in topical dose-response testing for new modes of action or new active ingredient products for mosquito

control allows for more rapid prototype assessments and provides better predictability of droplet penetration and performance in the field.

221 **Managing Saltmarsh Mosquitoes...Outside of the Saltmarsh**

Sarah McInnis, s.mcinnis@irmosquito2.org, Shawna James

Indian River Mosquito Control District (IRMCD) received a routine mosquito complaint in 2019. The findings, however, were not routine. We found two salt marsh mosquito species (*Aedes taeniorhynchus* and *Aedes sollicitans*) in the western part of our District, where there is no salt marsh habitat. Their habitat is brackish salt marsh which exists along our eastern coast. How did our salt mosquitoes end up so far west? We found the source of breeding on a commercial aquaculture farm adjacent to the site of the initial complaint. Saltwater discharges were occurring outside of their lined ponds resulting in creation of an artificial saltwater breeding ground for salt marsh mosquitoes. After two years of gathering data and providing suggestions to the aquaculture farm, the salt-marsh mosquito problem on the property persisted. IRMCD worked with multiple local and state regulatory agencies to solve the problem. This presentation will overview how the problem was identified, results of the data collection, potential solutions, and coordination efforts with other agencies to ensure problem was effectively remedied.

222 **Will history repeat itself? Could *Aedes aegypti* be re-displaced by *Ae. albopictus* from the re-established area in the City of Gainesville, Florida?**

Peter Jiang, jiangy1@cityofgainesville.org

The discovery of *Aedes aegypti* in the City of Gainesville, Florida in 2019 after a 26-year absence prompted Gainesville Mosquito Control program (GMC) to increase its *Aedes* surveillance. Starting from early 2020, GMC used multiple methods to monitor *Ae. aegypti* and *albopictus* population in the area (Pleasant Street Historical District, PSHD) where *Ae. aegypti* was detected first. For the first two years, less than 1% (0.45% in 2020 and 0.70% in 2021, respectively) adult *Ae. albopictus* was collected from the PSHD BGS traps. However, significantly higher number (>8.5%) of *Ae. albopictus* was collected from the same area in 2022. The percentage of *Ae. albopictus* vs. *Ae. aegypti* ranges from 0-30% in 2022, increasing as the time progress and reaching its peak at the month of August. Results show that *Ae. aegypti* is being replaced by *Ae. albopictus* in this newly established area. Will history repeat itself? Only time can tell.

223 **Evaluation of Deltamethrin-Impregnated Screening for Localized Reduction of Mosquitoes in Deployed Settings**

Jim Cilek, james.e.cilek.civ@mail.mil, Jason Fajardo, Josh Weston, Mark Mitola, Adam Salyer

From a Force Health Protection perspective, insecticide-impregnated screened panels placed along the perimeter of outdoor/semi-outdoor work areas may provide an additional layer of protection from mosquito vectors. Studies were conducted within a deciduous forest adjacent to a brackish needle grass marsh located at Kings Bay, GA. A 0.4% deltamethrin-impregnated screen material (with untreated screen as a control) was evaluated as open top screen cubes at heights of 3, 2.4, and 1.5 m by 3 m wide (n=4 each) at different time intervals from March 2019 through August 2021. Each

cubicle contained a CDC light trap with dry ice. Field results indicated that the majority of mosquitoes collected from all traps were *Aedes taeniorhynchus* and trapped mosquito counts in 3 m tall untreated cubicles (at 1 wk) were reduced considerably compared with sentinel CDC trap counts. One 3 m tall treated and untreated cubicle was retained as sentinels to monitor reduction by physical exclusion (if present) throughout the 107 wk study while other cubical heights were investigated. We found $\geq 80\%$ mosquito reduction occurred in 3 m tall deltamethrin-treated screen cubes on 13 of 107 weeks; with 9 time periods attributable to the insecticide ($P \leq 0.05$). In treated 2.4 m tall cubes, similar reduction occurred for 7 of 52 wks; the insecticide provided reduction at 4 of those time periods. In 1.5 m tall treated cubicles $\geq 80\%$ reduction occurred on 5 of 43 wks with insecticide providing reduction at 2 time periods. The deltamethrin screen produced 100% mortality in bioassays against laboratory-reared female *Aedes aegypti* throughout the study. Data suggests the majority of mosquito reduction in treated cubes was attributed to physical exclusion while the insecticide provided some reduction.

224 **Forecasting population trends of West Nile virus vector *Culex nigripalpus* using predictive modeling**

Robert Straser, rstraser@cmcd.org, Keira Lucas, Rebecca Heinig

Culex nigripalpus is one of the most important vectors of West Nile virus in southwest Florida. Due to the medical significance of the species, understanding the seasonal, long-term, and projected trends of *Cx. nigripalpus* is critical to reduce the risk of human infections. The use of statistical models have become increasingly important for forecasting infectious disease risk to guide public health. The Collier Mosquito Control District, located in Collier County, Florida, has curated an extensive database of spatial and temporal mosquito abundance, as well as operational control activities within the District since 2010. Using a combination of mosquito surveillance records and local climate data, we evaluated several modeling methods to optimize predictive forecasting of population trends of *Cx. nigripalpus*. This project highlights the prospects and limitations of using predictive forecasting to evaluate mosquito abundance trends, which may help the Collier Mosquito Control District perform more targeted treatments against *Cx. nigripalpus*.

Innovative Products and Technologies for Mosquito Control Symposium

225 **Contribution of Innovative Approaches for Sustainable Vector Control to Malaria Elimination in China**

Qiyong Liu, liuqiyongcdc@126.com

Malaria is a serious and fatal global vector-borne disease. It also caused a heavy disease, economic and social burdens in China. Following 70-year concerted efforts, China has been awarded a malaria-free certification by the World Health Organization (WHO) in June 2021. This paper summarizes the control strategies of *Anopheles* vectors as the innovative and stratified approaches for Sustainable Vector Control (SVC) to eliminate malaria from China. The SVC was adjusted according to the socio-economic status, environment and ecology, vector *Anopheles* species and malaria epidemic level. As for the post-elimination stages in China, the risk of imported malaria cases caused re-transmission and the challenges of *Anopheles* control must be emphasized. Sustainable and precise vector control is still

required during the post-elimination stage to consolidate malaria elimination achievements in the country. In addition, China's innovative vector control strategies, technologies and experiences may contribute to malaria control and elimination programs in other developing countries.

226 Larvicidal Efficacy of Sumilarv WSPs Deployed in Catch Basins

Caleb Corona, caleb.corona@mgk.com

The impact of vector-borne disease is felt across the world. Negative impacts of the spread of these types of diseases can be seen in areas where large human populations overlap with the presence of mosquito populations. As populations have grown and large precipitation events become more common, the need for water management has led to an increase in the number of catch basins being used to provide this necessary service. The presence of these basins provides a great environment for the establishment of breeding sites for mosquito populations in the field. Mosquito control professionals rely on larvicidal applications to treat catch basins that are producing mosquito populations. Catch basin applications of larvicides have displayed mixed results due to the complexity of biotic and abiotic factors associated with each individual environment.

In response to the need for catch basin mosquito control, MGK has developed a larvicidal product for treating problematic catch basins. Sumilarv 0.5G WSP, a product containing the insect growth regulator pyriproxyfen was developed specifically for deployment in catch basins. One of the main issues facing most larvicides is decreased efficacy of the product following major precipitation events or the introduction of organic matter into catch basins. Sumilarv WSPs have shown the ability to inhibit mosquito emergence from catch basins for up to 180 days after treatment. The extended efficacy of the product can be attributed to the nature of the active ingredient and the uniqueness of the formulation associated with the product. The overall efficacy of the Sumilarv WSPs paired with the single use method of application makes this product an extremely efficacious and cost-effective control option that can be deployed to control mosquito populations in catch basins.

227 Cloud based tool to swath characterize granular applications

Haley Johnson, haley.johnson@valentbiosciences.com, Leanne Lake, Katie Williams, Banugopan Kesavaraju

Long gone are the days of scribbling swath characterization calculations on paper and second guessing the accuracy of applications. This presentation will review the new easy-to-use swath characterization cloud application freely available on the Valent BioSciences webpage. Accurate swath characterization is essential for optimizing product efficacy, cost, and helps ensure applications are within label requirements. This user-friendly interactive app can be used for granular larvicides and allows applicators to conduct aerial swath analysis by simply uploading a single document. After uploading the appropriate data into the app, a downloadable report is generated visualizing both predicted and actual swath. Users can adjust swath width and application rate to visualize how these metrics in real time affect the swath characterization of the uploaded data. An overview of how to conduct aerial granular swath characterization analysis using the cloud-based app will be provided along with information on how to access the free tool.

228 The In2Care Mosquito Trap for precision mosquito management

Eva Buckner, eva.buckner@ufl.edu

The In2Care Mosquito Trap (In2Care trap) is a pyriproxyfen autodissemination station developed to control *Aedes aegypti* and *Aedes albopictus*, vectors of dengue, chikungunya, yellow fever, and Zika viruses. I have been collaborating with In2Care since 2015 to evaluate the efficacy of the In2Care trap in semi-field and field trials. This presentation will provide an overview of completed projects beginning with the initial semi-field evaluations of the trap against *Aedes aegypti* and *Aedes albopictus*, which were performed at Manatee County Mosquito Control District in southwestern Florida. The trap was found to be attractive to gravid *Ae. aegypti* and *Ae. albopictus* females. The gravid females that visited the trap were able to disseminate pyriproxyfen to surrounding water-filled containers, leading to mosquito emergence inhibition. Additionally, females in treatments with In2Care traps experienced reduced survivorship due to the impact of the *Beauveria bassiana* spores compared to control treatments. Next, a field study was conducted to compare the ability of the In2Care trap to control *Aedes aegypti* to integrated vector management techniques in Manatee County, FL. The In2Care trap only treatment was effective at controlling *Aedes aegypti* but required more time and labor compared to the integrated vector management techniques suggesting that using the traps alone may not be the best control strategy for large areas for programs with ground and aerial larviciding and adulticiding capabilities. However, it can be useful in hotspot areas or in within integrated vector management programs.

229 **Setting up barriers for *Aedes albopictus* in urban concrete jungles**

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Mosquito control programs in the United States are still searching for best management practices to control the Asian tiger mosquito, *Aedes albopictus* (Skuse; Diptera: Culicidae). Most intervention methods for this species are labor intensive or short term. In order to reduce the pressure of repeating inspections and treatments from mosquito operations, we investigated the effectiveness of barrier spray pesticide applications within urban and suburban residential yards in New Jersey as a control strategy using a before-after-control-impact (BACI) approach. We conducted barrier treatment with Suspend Polyzone (deltamethrin, 4.75% a.i, Bayer Environmental Science, Research Triangle Park, NC) in 2015 and 2016 and repeated in 3 wk to all accessible properties, and alleyways in the study site in Mercer County, New Jersey. Next we tested applications of Demand CSR pyrethroid (9.7% AI lambda-cyhalothrin) only or combined Demand CSR and Archer IGR insect growth regulator (1.3% AI pyriproxyfen) in Mercer and Hudson counties for 3 years during peak mosquito season (2016-2018). Suspend Polyzone applications proved efficacious, however lasted for 10 days. Demand CSR applications resulted in significant decreases in adult mosquito abundance posttreatment compared with the untreated control. The field study results were supported by laboratory no-choice bioassays using treated leaf foliage. While barrier treatments are a promising tool for mosquito management, it is important to remember in order to achieve effective mosquito control, variety of techniques such as barrier treatments, larval control, sanitation, source reduction, adult control, etc. should be combined into a complete integrated mosquito management program.

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

Atom Rosales, arosales@cmcd.org, Noe Pineda, Robert Straser, Keira Lucas, Rebecca Heinig

The BG-Counter is a remote surveillance tool that uses an infrared light sensor to quantify the total number of mosquitoes captured under field conditions. Every 15 minutes, the data are transmitted to a web-based interface, providing real-time insights into mosquito activity. Despite the BG-Counter's adoption by a number of mosquito control districts, little information exists on their performance in areas such as Collier County, which has a uniquely diverse and abundant population of mosquitoes. This study investigated BG-Counter accuracy at seven Collier County locations representing different mosquito habitats. Traps were outfitted with a collection bag and run overnight, and captured mosquitoes were sexed, counted, and identified to species. Because the BG-Counter relies partly on body size to distinguish mosquitoes from other organisms, non-mosquito arthropods were also identified and counted to assess their impact on the traps' accuracy. During the course of the study, over 30 of Collier County's 51 known mosquito species were captured by the traps, suggesting that the BG-Counter successfully sampled a broad range of the mosquito population. Further, the number of captured mosquitoes generally agreed with the automated counter data, indicating that the BG-Counter is a reliable indicator of mosquito activity in Collier County.

231 **Primary Uses of Drones in Mosquito Control and Becoming FAA-Certified**

Madeleine Schmitz, mschmitz@clarke.com

Primary Uses of Drones in Mosquito Control and Becoming FAA-Certified The role of drones, or unmanned aerial vehicles (UAVs), is becoming increasingly significant and widespread within mosquito control programs as they provide application flexibility, assistance in surveillance, and ease of operation. District and pesticide applicators must become licensed as FAA-certified drone pilots to utilize drones as part of a mosquito control strategy. Given the different requirements for first-time and current pilots, as well as the variety of topics that must be studied, the path towards licensing may sound daunting. This session will review the basics of drones used for mosquito control, detail the knowledge necessary to become a drone pilot and outline a clear and simple process for obtaining an FAA drone license.

232 **Updates and Improvements to Caged Mosquito Field Trials**

Kattie Morris, kmorris@clarke.com, Kattie Morris, Derek Fields, Yemi Bullen-McClain, Victoria Hyrczyk, Madeleine Schmitz, Mackenzie Wilson

Updates and Improvements to Caged Mosquito Field Trials Using small mesh cages to evaluate mosquito adulticide products has been a tool employed by mosquito control districts and industry alike for several years. However, much of the equipment used for these trials is homemade, and there is wide variability in the tools and protocols used. Recently, new research has allowed for streamlining the process of conducting these trials, and advances in 3D printing have made creating the necessary equipment more precise and user-friendly. This session will share field testimony of the impacts of innovations resulting from years of collaboration between industry and mosquito control districts.

233 **Laboratory and Field Evaluations on a Novel S-methoprene-based Product Line OmniPrene™**

Tianyun Su, stevensu1995@gmail.com

To face the challenges of emerging and resurging mosquitoes and mosquito-borne diseases, research, and development on novel larvicidal active ingredients or innovated formulations is critically important, particularly when we are at historical low in environmentally friendly products. A new product line OmniPrene™, including 4.25% granules, and its extended products water-soluble pouch (WSP) and extended WSP (XWSP), as well as microencapsulated suspension (CS) has been developed, evaluated, and registered with the United States Environment Protection Agency (US EPA). The granular formulation provided over 90% efficacy against *Aedes* and *Anopheles* for at least 49 days, and against *Culex* for up to 42 days at 2.5-10 lb/ac when water depth was 12-in. The control levels were further elevated when water was 6-in, where over 90% control was observed for 63 days against *Aedes* and *Anopheles*, but 56 days against *Culex*. The WSP and XWSP were customized for persistent production sources such massive urban storm water drainage systems with over 90% control for 105 and 266 days respectively against the southern house mosquito *Culex quinquefasciatus* in simulated catch basins at 1 pouch/basin. As to the liquid formulation OmniPrene 20CS, it provided greater than 90% control for 14-21 days against *Aedes aegypti*, at least 21 days against *Anopheles hermsi* and up to 14 days against *Culex quinquefasciatus* when it was applied at the label rate of ¾ and 1 OZ/ac. It is anticipated that this new product line will add to the new arsenals in combating mosquitoes of public health concern.

Operations

234 **Impoundment Management: An Historical Method of Source Reduction**

Sherry Burroughs, s.burroughs@irmosquito2.org

Controlling salt marsh mosquitoes has been a challenge in Florida since the 1920s. Efforts to eliminate the salt marsh mosquitoes began with draining, ditching and filling. In the 1950s, construction began on the creation of mosquito control impoundments within the salt marshes along the east coast. Impoundments were created by constructing earthen dikes around the marsh to isolate the high salt marshes and mangrove swamps from the adjacent estuary. Environmental concerns raised in the late 1970s brought about the creation of the Subcommittee on Managed Marshes (SOMM), which was a subcommittee of the Florida Coordinating Council on Mosquito Control (FCCMC). The FCCMC is an advisory group that is responsible for providing review and comment on saltmarsh management plans. Over the years, SOMM established guidelines for impoundment management and through several research efforts, developed the Rotational Impoundment Management technique, known as RIM. The presentation will overview the history of impoundments and the challenges, both old and new, of managing these salt marshes.

235 **Optimizing Forecasts for Sustainable Mosquito Control**

Jason Farned, jfarned@sgvmosquito.org

Efficient control of mosquitoes and elimination of habitat is critical to protecting the public from disease and nuisance. Strategies that follow integrated vector management (IVM) best practices are frequently used, which can guide allocation of operational resources. However, due to rapid changes during the season and unexpected responses to public health, agencies may find it difficult to quickly shift and track resources. The San Gabriel Valley Mosquito and Vector Control District aims to improve operational efficiency through accurate forecasting and targeted resource allocation.

Utilizing Power BI to analyze key activity and timing data from field staff, the operations department is now able to visualize seasonal work patterns and peak drivers of demand, build best practices based on efficiency, create reasonable expectations and timing standards, and accurately forecast future demand for resources.

This presentation will discuss the process of building and applying an innovative new forecasting tool, and it's positive impact to efficiency, efficacy, and moral.

236 Green Pool Detection and Response: Changing Methods with Evolving Technology

Dan Fisher, dfisher@fightthebite.net

In 2007 with West Nile virus on the rise, the District purchased aerial imagery for the first time to search for unmaintained "green" swimming pools. In the 15 years since that first flight, nearly every part of the process has changed, using new technology to increase efficiency in time, cost, and compliance. With the latest imaging methods, artificial intelligence software and GIS automation processes, the District has developed new workflows in detecting, responding to, and managing the ongoing problem of green pools. This talk will examine the history of the District's approach and look ahead to continued evolution with the use of artificial intelligence.

237 PCR Lab Capabilities and Impact on Control and Applications Decisions

Lauren Lavezzi, LLavezzi@clarke.com

PCR Lab Capabilities and Impact on Control and Applications Decisions Mosquito control companies and programs have traditionally used RAMP testing to evaluate local populations for vector disease. However, RAMP testing consists of an involved sample prep and test process and requires confirmation through a third-party PCR lab. All in all, RAMP testing can take up to 14 days, depending on the capabilities of the outside lab. Clarke's surveillance team opted to set up PCR lab testing in their facilities to quicken and control the testing process while obtaining more sensitive test results. This session will share the experience of the surveillance team tasked with establishing internal PCR testing, including best practices, challenges faced, and how these faster, more accurate test results impacted mosquito control and application decisions.

238 A Case for Cohabiting: Mosquito Control and Endangered Species Can Live Together

Michelle Selander, mselander@clarke.com

A Case for Cohabiting: Mosquito Control and Endangered Species Can Live Together Increased regulatory and media scrutiny around pesticide exposure, habitat threats and climate change are all contributing to a heightened sensitivity to human impact on beneficial insects and protected or endangered species. While happening on a macro level, we see and feel the impact directly in public

health mosquito control when community residents, advocacy groups, and even regulatory or legislative bodies challenge the merits of mosquito control practices with concerns about sensitive, threatened, or endangered species habitats. The goal of this session is to share best management practices from a beneficial insects perspective for integrated mosquito management programs, drawing from several years of operational work performed in Illinois, including in areas where an endangered species (the rusty patched bumble bee) is present. This talk will focus on the criticality of collaborative relationships with local/state regulatory agencies and responsible stewardship that extends beyond the basic label requirements to build community trust and support for mosquito control focused on public health benefits.

239 Advances in automated identification and pooling technology

Hanan Lepek, Hanan@senecio-robotics.com

Mosquito abatement districts performing adult surveillance face challenges of increasing labor costs in parallel to shortage in experienced personnel. Repeatedly, majority of mosquito traps contain the same few common mosquito species, while still the technicians process manually each one of the insects, as they encounter the non-common species from time to time.

A semi-automation technology for combining the advantages of having human in the loop as a content expert together with artificial intelligence and robotics can provide a powerful tool for enhancing the surveillance team performance by an order of magnitude.

In the presentation, newest advances in robotic processing of mosquitoes will be shared, together with demonstrating how lab technicians work can be boosted to a whole new level with the introduction of new automation tools. Challenges of the development phase along with latest real-world results will be discussed. Join the presentation and stay updated with latest advances in robotics in the field of mosquito surveillance.

240 Operational LiDAR use at Lee County Mosquito Control District

Laura Mattas, mattas@lcmcd.org, Aaron Lloyd, Nick Lefkow, Durrell Hagood, David Hoel

The Lee County Mosquito Control District (LCMCD) manages the mosquito population throughout Lee County, Florida by utilizing classic Integrated Mosquito Management (IMM) techniques, and incorporating emerging technologies that can provide additional effective and efficient treatment options for pestiferous and disease vector mosquitoes. In 2022 LCMCD acquired a light detection and ranging (LiDAR) system for use in habitat determination and polygon verification concerning aerial and ground larviciding missions. LCMCD recognizes the potential for various applications of LiDAR data in mosquito control. LiDAR is a powerful remote sensing technology that collects precise three-dimensional data of surface features, which are classifiable based on sensor characteristics, and can be used to generate elevation models with overlaying vegetation and infrastructure removed to reveal previously obscured bare earth and waterbodies. Primary objectives for this technology at LCMCD include; locating cryptic breeding locations within aerially obscured regions too densely vegetated to inspect thoroughly, predicting breeding habitats based on high-resolution elevation models and corresponding tidal/rain data, and change detection studies in ambiguous and dynamic habitats that pose significant challenges to aerial visual inspection. The current implementation of LiDAR includes

conducting habitat research and refining treatment polygons to provide more prescribed treatments to breeding locations. Despite being in its infancy, LiDAR Operations at LCMCD have improved aerial larviciding missions and are projected to accumulate enough potential savings to cover the cost of the initial investment.

241 **Making a case for Bio-controls: differentiating inundative versus classic strategies with the native entomopathogenic nematode, *Romanomermis culicivorax***

Anita Schiller, aschiller@hcp4.net

Vector suppression and control utilizing whole organism biocontrol agents appear to be a thing of yesteryear. Should we shelf these strategies for good and move on to conventional control methods without looking back? We make the case to say: not so fast! and unpack classic versus inundative strategies using *Romanomermis culicivorax* and share the production process to incorporate this unique biocontrol agent into integrated control operations.

242 **Teamwork, tech, and taxes to tackle tidal mosquitoes**

Sarah Lawton, sarah@mosquitoes.org, Michelle Robles, Mark Wieland, Joseph Huston, Ryan Clausnitzer, Eric Haas Stapleton

In the absence of effective control efforts, *Aedes dorsalis* can be highly abundant in salt marsh habitats and fly en masse to harass nearby communities. Exceptionally high tides trigger *Ae. dorsalis* eggs to hatch and warm summer weather accelerates larval development, producing biting adults often within a week. The one-week window of opportunity to apply larvicide coupled with challenges accessing remote sites in the salt marsh and allocating large numbers of staff to apply larvicide by foot can limit the efficacy of controlling this mosquito. We utilized an A1 mist sprayer (MS) to loft large quantities of liquid Vectobac 12AS larvicide to control *Ae. dorsalis* after exceptionally high tide events. We analyzed the number of acres that were treated, the time it took staff to inspect and treat the sites, the cost of the products used, the cost of the staff utilized, the average larvae per dip, and the abundance of *Ae. dorsalis* in nearby EVS traps in the years before and after the MS was brought into our program. More acres were treated, and more staff time was spent treating at the sites after the MS was implemented in our control program. The cost analysis of products used during the two time periods shows that we spent similar amounts of money on larvicides, but fewer larval and adult mosquitoes were found after the MS was implemented. By combining treatments via MS with hand treatments, we were able to achieve comprehensive larvicide applications that effectively controlled *Ae. dorsalis*. By building mechanization and automation into our control program, we are better poised to address future challenges, such as the anticipated arrival of invasive *Aedes* in our county, without needing to increase staff substantially.

Adult Control II/Systematics

243 **Mosquito Fauna (Diptera: Culicidae) Of Rawalakot Azad Jammu and Kashmir, Pakistan**

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Mosquitoes are cosmopolitan insects, and they transmit many infectious parasites like viruses, protozoan, nematodes, and bacteria and act as vectors of several diseases such as malaria, chikungunya, filariasis, and dengue fever throughout the world. Study the mosquito born disease specimens were collected from different localities of Rawalakot, Azad Jammu and Kashmir. Collected specimens were identified into nine species belonging to five genera, under two sub-families. All nine identified species are reported for the first time from studied area. Among them, three species *Aedes koreicus* (Edwards, 1917), *Armigeres kesseli* Ramalingam 1987, and *Coquillettidia Tenuipalpis* (Edwards, 1924) are new record from Pakistan.

Keywords: Anophelinae, *Aedes*, *Armigeres*, Culicidae, Culicinae, *Culex*, Mosquito, Fauna

244 **Discovery of a New Black Fly Species *Simulium ustulatum* (Diptera: Simuliidae) from San Joaquin County, California, USA**

Shaoming Huang, shuang@sjmosquito.org, Peter adler

A new black species was discovered and described by using an integrated taxonomic approach that included traditional morphological identification, DNA and chromosomal barcoding. The new species belongs to the *Simulium* (*Boreosimulium*) *annulus* group and is assigned the formal name *Simulium ustulatum* n. sp. The female of this species is morphologically inseparable from other members of the *S. annulus* group, but the male and larva are structurally unique. DNA barcoding using cytochrome c oxidase I (COI) gene confirmed its relationship with members of *S. annulus* group. Chromosomal barcoding revealed this new species has the most rearranged chromosomes of all North American members of *S. annulus* group and differs most conspicuously by having a large chromocenter. The new species is the only western North American member of the *S. annulus* group that inhabits low-elevation, disturbed landscapes.

245 **Incorporating In2Care® Mosquito Trap into IPM toolbox to control invasive *Aedes* mosquitoes: Lessons from the West Valley region of the San Bernardino County**

Jennifer Castellon, jthieme@wvmvcd.org, Solomon Birhanie, Mayra Macias, Michelle Brown

Aedes aegypti is the predominant mosquito species that could potentially transmit dengue, chikungunya, yellow fever, and Zika viruses. Due to the widespread distribution of invasive *Aedes* mosquitoes in recent years across California, the risk of *Aedes*-borne diseases has become a public health threat. Currently available Integrated Pest Management (IPM) strategies have not been effective to control these mosquitoes because of their cryptic breeding habitats. Thus, there is a crucial need for innovative control measures to be added in to the existing IPM toolkit to deal with these urban mosquitoes. At the West Valley region of the San Bernardino County, In2Care® Mosquito Trap have been used as part of our IPM to control *Aedes* mosquito population since 2019. Here we report summary of field data to determine effectiveness of these traps to control invasive *Aedes* populations. First, exploratory trapping was conducted across our District by setting BG Sentinel traps in over 200 houses. Then, based on *Aedes* mosquito counts, a total of 24 high count sites were recruited across the District for this study. In2Care® Mosquito Traps were placed at these sites at the beginning of the mosquito season and kept until the season abated. Every month BG Sentinel traps were set to monitor the *Aedes* population at these sites. To serve as controls, BG Sentinel traps were also set in neighboring

houses within ¼ mile from the sites with In2Care® traps. Trapping continued from 2021 to 2022. The results indicated that there was significant reduction in *Aedes aegypti* mosquito population that ranged from 67.5% to 90.3% compared to sites without the In2Care® traps. Overall, our findings implicate that incorporating In2Care® Mosquito Traps into our existing IPM toolkits is beneficial to control invasive *Aedes* mosquitoes in California.

246 **Preparation for targeted sterile insect technique to control invasive mosquitoes in California: mosquito colonization, gender emergence ratio and separating the males**

Solomon Birhanie, sbirhanie@wvmvcd.org, Jennifer Castellon, Mayra Macias, Michelle Brown

The urban-adapted, daytime-biting *Aedes aegypti* is the primary mosquito vector of dengue and has the potential to transmit several other arboviruses, including Zika, chikungunya, and yellow fever. In California, *Ae aegypti* has spread in over 300 cities within 22 central and southern counties in less than a decade. Due to its cryptic breeding habitats, control efforts have not been successful so far. Therefore, there is a critical call for innovative biological tools such as Sterile Insect Technique (SIT) to effectively control invasive mosquitoes by expanding the existing integrated pest management (IPM). Here, we provide a summary of laboratory experiments conducted to establish *Ae. aegypti* colony and examine gender emergence ratios and strategies to separate males for SIT. The use of pressure operated flasks with added yeast yielded an over 80% egg-hatching success in 1.5 hr while hatching without yeast resulted below 50% hatching success. Pupae were monitored for adult emergence in plastic, and males were manually separated immediately after emergence. Among freshly emerged adults, the proportion of males was highest in the first 24 hrs (78%) and dropped to 13% on Day 6. Implications of these findings and future research ideas are discussed.

247 **Multi-centre discriminating concentration determination of broflanilide and potential for cross-resistance to other public health insecticides in *Anopheles* vector populations.**

Louisa Messenger, louisa.messenger@unlv.edu

Novel insecticides are urgently needed to control insecticide resistant populations of *Anopheles* malaria vectors. Broflanilide acts as a non-competitive antagonist of the gamma-aminobutyric acid (GABA) receptor and has shown prolonged effectiveness as an indoor residual spraying (IRS) product (VECTRON™ T500) in experimental hut trials against pyrethroid-resistant vector populations. This multi-centre study expanded upon initial discriminating concentration testing of broflanilide, using six *Anopheles* insectary colonies (*An. gambiae* Kisumu KCMUCo, *An. gambiae* Kisumu NIMR, *An. arabiensis* KGB, *An. arabiensis* SENN, *An. coluzzii* N’Gousso and *An. stephensi* SK), representing major malaria vector species, to facilitate prospective susceptibility monitoring of this new insecticide; and investigated the potential for cross-resistance to broflanilide via the A296S mutation associated with dieldrin resistance (*rdl*). Across all vector species tested, the discriminating concentration for broflanilide ranged between $LC_{99 \times 2} = 1.126 - 54.00 \mu\text{g/ml}$ or $LC_{95 \times 3} = 0.7437 - 17.82 \mu\text{g/ml}$. Lower concentrations of broflanilide were required to induce complete mortality of *An. arabiensis* SENN (dielddrin-resistant), compared to its susceptible comparator, *An. arabiensis* KGB, and there was no association between the presence of the *rdl* mechanism of resistance and survival in broflanilide bioassays, demonstrating a lack of cross-resistance to broflanilide. Study findings provide a

benchmark for broflaniide susceptibility monitoring as part of ongoing VECTRON™ T500 community trials in Tanzania and Benin.

248 Exposure of dengue-1 virus to Aedes aegypti and sensitivity to adulticides

Robert Aldridge, robert.aldridge@usda.gov, Barry Alto, Roxanne Connelly, Bernard Okech, Blair Siegfried, Kenneth Linthicum

Chemical control of adult vector mosquitoes relies on effective evaluation of active ingredients to determine their performance on healthy vector populations. However, chemical control is frequently used to quell potential disease outbreaks from infected adult mosquitoes. Evaluation of active ingredients of chemical controls to pathogen exposed mosquitoes has not been performed and therefore the condition of mosquitoes utilized in bioassays evaluating active ingredients is flawed. Here we evaluate LD50 difference to pathogen exposed mosquitoes and non-exposed mosquitoes to determine if prior pathogen exposure influences insecticide susceptibility.

249 Balancing the Benefits of Urban Development and Rural Agroecosystems with Public Health - the Continuing Saga of Insecticide Resistance

Deborah Dritz, ddritz@fightthebite.net, Sarah Wheeler, Gary Goodman

Insecticide resistance in insect populations has been characterized as existing in a checkerboard pattern geographically. This is particularly problematic for public health professionals when a block of resistance occurs in high mosquito producing habitats. Identification of those habitats and characterizing the presence or absence of resistance is critical for a successful vector control program. The Sacramento-Yolo Mosquito Abatement District, located in the Central Valley of California encompasses 2,013 square miles which includes the capital city of Sacramento as well as many urbanized areas and, even in a drought year, 17,225 acres of rice. The results of our insecticide resistance detection and monitoring program using the CDC bottle bioassay will be presented with a focus on how urban micro habitat, rural crop practices and control product options can affect programmatic outcomes.

250 Efficacy of Ground ULV Adulticide Application: A Multi-Year Study in the Chicago Suburbs

Kristina Lopez, kalopez@wisc.edu, Patrick Irwin, Susan Paskewitz, Lyric Bartholomay

Documentation that adult mosquito control practices change Culex populations evidenced by entomological outcomes or epidemiological outcomes is inadequate and often contradictory. It is imperative to understand how adulticide applications interact with female mosquitoes at different gonotrophic stages so that a more targeted and efficacious approach can be utilized. We evaluated the impacts of multiple adulticide applications on Culex pipiens and Cx. restuans abundance, age structure, and West Nile virus infection over multiple years. Both host-seeking and gravid mosquitoes were collected daily from 24 residential properties, half of which were located within three large, treated sites. Adulticide was sprayed once per week for five sequential weeks during peak Culex abundance periods. Zenivex E20 and Anvil 10+10 were both used for adulticide, though resistance to both products was discovered. Analysis and WNV testing is on-going, though preliminary results show no reduction in mosquito abundance and a rebound effect several days later. We observed a significant

decrease of parous mosquitoes in treated sites, reducing the risk for human WNV infection. Surprisingly, *Cx. pipiens* and *Cx. restuans* did not respond in the same way to adulticide application, highlighting the importance of differentiating the two species when possible.

Disease/Vector Studies I

251 Optimizing Trap Placement to Predict West Nile Virus Cases

Rebecca Smith, rlsdvm@illinois.edu, Anwesha Chakravarti, Bo Li, Dan Bartlett, Patrick Irwin

How do you decide where to place a mosquito trap for West Nile Virus (WNV) surveillance? And what makes a good trap, anyway? We will present a statistical approach for determining the value of a gravid trap in predicting human WNV cases in the next two weeks within a 1.5km radius of the trap. We will then use that value to show what landscape, infrastructure, and socioeconomic factors are correlated with the ability to predict when human cases will occur, and when they will not, within the Northwest Mosquito Abatement District in Cook County, Illinois. This approach will enable resource-limited mosquito control programs to identify better locations for their trap-based surveillance.

252 Development of Mosquito Surveillance System with Artificial Intelligence (AI) Techniques

HEEIL LEE, isak@korea.kr, Sun Ran Cho, Gi-Hun Kim, Sang-Joon Lee, Byoung-Kwan Cho, Dong-Sik Ryu, Rong-Jin Jung

To management of mosquito-borne disease, mosquito surveillance including identification and population density is very important to decide vector control strategy. However, that is very time consuming work and necessary special knowledge. Because of that reason, we try to development mosquito surveillance system using artificial intelligence (AI) technique for proper and well-timed mosquito surveillance data. To make a mosquito image, we develop several devices with mosquito attraction, collection, freezing, and take a photograph. For the mosquito identification, we trained image classification algorithm with dominant mosquito species including *Culex pipiens* complex, Anopheline, *Aedes albopictus*, *Aedes vexans*, *Culex tritaeniorhynchus* and non-mosquito species. This architecture is built by integrating the Swin-transformer backbone with the Fast R-CNN. The classification accuracy of single mosquito individual was 97.2%. After the classification, the result was transmit to internet server with the identification result and images. This novel mosquito surveillance system has conducted a field inspection. With these monitoring system, we can identify the proper mosquito control threshold and time.

253 Designing an Artificial Blood Meal (ABM) for the Feeding of *Aedes aegypti*

Alexander Weaver, alexanderweaver@ufl.edu, Nagarajan Ramasamy Rajagopal, Christopher Batich, Roberto Pereira, Philip Koehler, David Schechter

Mosquito-transmitted diseases such as Zika, West Nile, Chikungunya, dengue, and malaria severely threaten global health. Research into mosquito-borne illnesses and rearing for sterile

insect technology poses a serious challenge because fresh blood-feeding to rear female mosquitoes is difficult from logistical, ethical, economical, and safety perspectives. A new meal has been identified; an aqueous solution for feeding the disease vector *Aedes aegypti* that does not contain liquid blood (an artificial blood meal; ABM) has been developed to reduce the challenges associated with transporting and storing blood for rearing purposes. The novel component of this meal is dried porcine blood meal that is hypothesized to contain a preserved phagostimulant, which promotes mosquito feeding. Additional ingredients in this ABM formulation include phosphate-buffered saline as a solvent and a commercial-grade milk protein. The dry meal is shelf-stable and can be held at room temperature. This ABM encourages oogenesis as engorged mosquitoes have been shown to produce viable eggs under similar hatching and rearing conditions as fresh blood-fed mosquitoes. Additionally, the dry mixture can be produced at a significantly lower cost than that of defibrinated bovine blood, which is the standard for mosquito feeding. The dry mixture does not require expensive phagostimulants like adenosine triphosphate (ATP) or refrigerated, proteinaceous whole-blood derivatives.

254 **The Dance of Two Agencies: Local Mosquito Abatement Program and Local Health Department's response to Potential Imported and Locally Acquired Mosquito-borne Disease Cases.**

Milton Sterling, sterling@lcmcd.org, David Hoel, Aaron Lloyd

Mosquito-borne diseases are considered among the world's most deadly illnesses known to man, and continue to pose a significant public health risk. The collaborative partnership and cooperation between local mosquito control districts and health department agencies plays an important role to protect the public from imported and locally acquired mosquito-borne diseases.

Due to Lee County's ever-changing demography of residents and visitors traveling to and from Central, South America and the Caribbean, there have been a constant influx of travel-related exotic mosquito-borne illnesses that are currently among the existing non-exotic arbovirus illnesses already being monitored. Lee County Mosquito Control District have developed a synergetic relationship with the local health department for decades, serving on the frontline and working closely together to monitor potential human cases and competent vectors that may cause mosquito-borne disease outbreaks. This presentation will discuss the distribution of information between both agencies, and the procedure surrounding activated responses regarding potential human arbovirus cases.

255 **West Nile Virus Surveillance in Sentinel Chickens and Mosquitoes in Panama City Beach, Florida, from 2014 To 2020**

Michael Riles, mriles@central.com, Eddie Summers, Bryan Giordano

Over 20 years since its introduction, the West Nile virus (WNV) continues to be the leading cause of arboviral disease in the USA. In Panama City Beach (Bay County, FL), WNV transmission is monitored using sentinel chickens and testing mosquito pools for presence of viral RNA. In the current work, we monitored WNV transmission from 2014 to 2020 through weekly serology sampling of sentinel chickens; mosquito populations through biweekly mosquito collections by suction traps (1 m and 9 m) and weekly gravid trap collections; and mosquito infection rates using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Samples were sent to the Bureau of Public Health Laboratories (Tampa, FL) for testing presence/absence of WNV via RT-PCR assay. Our results indicated

that canopy surveillance could augment ground collections, providing greater proportions of *Culex* mosquitoes with less bycatch compared with ground collections. Serology indicated 94 seroconversions to WNV in the study area from 2014 to 2020. The most active year was 2016, which accounted for 32% (n = 30) of all seroconversions reported during the study period. We detected 20 WNV-positive mosquito pools from *Culex quinquefasciatus* during 2014-17; mosquito infection rates ranged from 2.02 to 23.81 per thousand (95% CI). Climate data indicated anomalously high precipitation in 2014-19 preceding WNV transmission. Data analyzed herein indicate utility in year-round continuous and diversified surveillance methodologies. This information is needed to properly calibrate future models that could assist with predicting transmission events of WNV in Panama City Beach, FL.

256 **Aging Field-Collected *Aedes aegypti* from the Florida Keys**

Catherine Pruszyński, cpruz@keysmosquito.org

Aedes aegypti is an important vector of many viruses that affect human health, and is the species responsible for the autochthonous dengue outbreaks in Key West in 2009 and Key Largo in 2020, FL, USA. Before transmission can occur, a virus must undergo an extrinsic incubation period within a mosquito. For dengue, this period can last between 6-14 days. Therefore, mosquito abatement efforts should focus on targeting the mosquitoes old enough to transmit the virus. Yet, aging mosquitoes is a difficult task and few methods exist to chronologically age mosquitoes. In this experiment, we used transcriptional profiling to measure the changes of two age-dependent genes, Sarcoplasmic calcium binding protein 1 and Aquaporin-11, within a known age group of *Ae. aegypti*. We used the known age group to develop a model that could predict the ages of field-collected mosquitoes, and divide them into three epidemiological significant categories: pre-EIP (1-5 days old), within-EIP (6-14 days) and post-EIP (15+ days). Mosquitoes were collected from October through November 2020 during the end of the Key Largo dengue outbreak. Our results indicated that at least half of the mosquitoes collected from Key West, Marathon, and Key Largo could be old enough to survive through the dengue EIP. These data provide baseline mosquito longevity in a post-outbreak situation, and elucidates potential future outbreaks as it pertains to mosquito survival. Age grading field-collected mosquitoes via transcriptional profiling can provide public health and mosquito control agencies with information to help them to better understand a vector population's capacity to transmit dengue.

257 **Microplastic ingestion alters the bacterial microbiota of *Aedes aegypti* and *Aedes albopictus***

Corey Brelsfoard, corey.brelsfoard@ttu.edu, Carla-Cristina Edwards, Gabriella McConnel, Yaizeth Gurrola-Mares, Jaclyn Canas-Carrell

Plastic pollution remains one of the most ubiquitous and menacing ecological threats worldwide. While larger plastics cause concerns when ingested by animals, little is known about the effects of much smaller microplastics, which can be formed by the degradation of plastics by sunlight, abrasion, and tire wear. Microplastics of a few microns in size or less are challenging to remove from the environment and are easily ingested by animals, mainly invertebrates, yet their biological effects are unclear. Microplastics have also been found to alter the gut microbiota of honeybees, collembola, and other soil-dwelling invertebrates resulting in gut dysbiosis. Here we demonstrate the presence of microplastics in artificial containers that make up mosquito larval habitat. Using fluorescent microscopy, we also show the ingestion of 1 μ m polystyrene microplastic beads by *Aedes aegypti* and *Aedes*

albopictus larvae. Results suggest there is little effect of ingestion of microplastics on adult emergence rates and adult survivorship of *Ae. aegypti* and *Ae. albopictus*. However, we did observe gut damage associated with the ingestion of microplastics. We also demonstrate that ingestion of microplastics results in dysbiosis of the bacterial microbiota but has little impact on the fungal microbiota composition in *Ae. aegypti* and *Ae. albopictus*. We discuss these results in the context of potential effects on mosquito fitness and vector competence of arboviruses.

258 **West Nile Virus in Tangipahoa Parish, Louisiana: Past and Present Mosquito Activity**

Colby Colona, colbycolona@gmail.com

Tangipahoa Mosquito Abatement was first established in 2003. The year 2022 was the most active year for West Nile virus in the history of our district. This talk will discuss mosquito data that was collected throughout our district during this time and compare to previous years using maps and graphs.

259 **Dirofilaria immitis in invasive Aedes and native Culiseta species from Orange County, California**

Daisy Rangel, dfrangel@ocvector.org, Brenda Velis, Julia Ly, Kassandra Reyes, Tara Thiemann, Phillip Spinks, Amber Semrow

Dirofilaria immitis (*D. immitis*), a filarioid nematode that causes dog heartworm infection, is transmitted by the bite of an infected mosquito. The number of heartworm cases in dogs and cats continues to increase nationally and this increase has been observed in both areas historically considered to be endemic for dog heartworm, as well as in areas of low endemicity like in Orange County, California. Increased disease cases can be attributed to the increase in mosquito populations and with the introduction of the vector competent *Aedes* mosquito species in Orange County, the purpose of this study was to test the prevalence of *D. immitis* positive mosquitoes in *Ae. aegypti*, *Ae. albopictus*, and *Ae. notoscriptus* as well as the native *Culiseta incidens*. Various traps were used to collect and test mosquitoes for 2020-2022 and *D. immitis* was detected in all four mosquito species. Positive dog heartworm infection rates in mosquito samples tested increased every year at 0.55% (2020), 1.28% (2021), and 1.75% (2022). All infected samples were collected starting in late June through November. Real-time PCR probes were used to detect the mitochondria cytochrome I (CO1) gene and *D. immitis* positive mosquito samples were confirmed by DNA sequencing. Our results reveal the presence of *D. immitis* in wild mosquitoes in Orange County, and based on the geographical distribution of the positives, the results demonstrate the need to continue to survey for this pathogen throughout the county as well as inform and educate veterinarians, animal care agencies, and the affected communities.

Larval Control/New Product Trials I

260 **The runnel concept, from past practice to expansion and recent application for marsh restoration, wildlife conservation and perhaps to mitigate climate change.**

Patricia Dale, p.dale@griffith.edu.au

Runnels, shallow spoon shaped channels, were first developed in SE Australia (Queensland and NSW) in the mid 1980s for the primary purpose of controlling salt marsh mosquitoes, thereby reducing reliance on larvicides, but also minimising impacts on the saltmarsh. The method was designed to follow the local topography and increase tidal flushing but not to drain saltmarshes. Runnels are a minimal form of Open Water Marsh Management source reduction, as it was implemented in the 1960s in eastern USA. At first there was curiosity about the method in the USA and then interest was shown in the early 1990s, with runnelling included in a range of source reduction methods. Subsequently, the runnel concept has evolved and its objectives have expanded to embrace salt marsh restoration and to protect endangered species habitats. More recently, faced with increasing sea level rise in NE USA, the method has been adapted aiming to assist in marsh restoration, while also controlling mosquito larval populations. There is currently also interest in Europe in runnelling to reduce the mosquito control related disturbance to wildlife, both on the ground and from the air.

261 **GLOBE Observer Mosquito Habitat Mapper: Mobile app surveillance and mitigation of larval habitats by citizen scientists**

Russanne Low, rusty_low@strategies.org, Cassie Soeffing, Peder Nelson, Dan Killingsworth, Dan Bartlett

In backyards and communities worldwide, citizen scientists support mosquito control efforts. NASA GLOBE Observer Mosquito Habitat Mapper is a free mobile app that connects the public with scientific researchers, mosquito management professionals, and policymakers. The app encourages users to identify and report standing water habitats and remove them from use as mosquito breeding sites. Users document their observations by photographing the larvae and habitats they find. In an optional step, users report the surrounding land cover and land use with photographs. With each submitted observation, they are supporting future vector modeling capabilities with satellite data. Mosquito Habitat Mapper data are shared in an open database for use by scientific researchers, mosquito management professionals, and educators. These data are also included as part of the citizen science data accessible through the Global Mosquito Observations Dashboard (GMOD).

We introduce the app and provide examples of professional mosquito control collaborations, research projects that use this data, and access to high-quality educational resources that are freely available for anyone in public outreach programs. With more than 34K data submissions, GLOBE Observer Mosquito Habitat Mapper is now available in 11 languages in 127 countries.

262 **Natular DT (Spinosad, Clarke) control dynamics in an artificial yard drain system**

Mario Novelo Canto, mnovelocanto@fightthebite.net, Steven Ramos, Daniela Arce, Sarah Wheeler

Larvicide products are widely used in vector control operations to target the immature stages of mosquitoes. However, aquatic mosquito habitats often include cryptic sources such as: septic tanks, planters, storm drains, and yard drains. These locations can be challenging to treat; therefore, effective and efficient treatment methods are essential. Natular DT (Clarke, IL.) is a compact bi-layer tablet with spinosad (*Saccharopolyspora spinose*) as an active ingredient. This product can be used in containerized and cryptic water sources to prevent mosquito development and emergence. The first

effervescent dose provides control within the first 24hr and the second dosage dissolves at a slower rate, providing continuous control for up to 60 days. At the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD), we tested the efficacy of Natular DT in an artificial yard drain system that resembled a typical suburban drain system. Larval mortality was assessed weekly and we found that the point of product application (inlet, outlet) affects the overall larval mortality dynamics, and that application in a single drain has cascading mortality effects in the rest of the interconnected drains. Our results suggest that Natular DT can be used as an effective control tool by SYMVCD as well as other abatement programs.

263 **Simple, small and smart system for monitoring and control of dengue vector**

Unsar Naeem-Ullah, naeem1130@yahoo.com, Syed Farooq Shah, Shafia Saba, Sikander Ali, Shafqat Saeed, Tariq Butt

Onset of dengue epidemics in Pakistan has now been a regular phenomenon. Management of the vector mosquito is sole method to control dengue as no vaccine is available. Vectors are generally controlled by synthetic pesticides which have many associated harms like insecticidal resistance, environmental pollution and threat to non-targets etc. Use of biocontrol agents such as entomopathogenic fungi can be effective alternate controlling measures.

So, a smart control device / system comprised on attract, capture and kill strategy was designed and tested in lab and also in field on small scale. The studies included evaluation of different chemicals as mosquitoes' attractants, design of low-cost plastic bottle-based trap and insect pathogenic fungus *Metarhizium brunneum* as biocontrol agent against *Aedes aegypti*.

Of the different chemicals tested, ammonia appeared to be most effective in attracting *Ae. aegypti* under controlled conditions. Four different designs were evaluated for a plastic bottle-based trap. The bottle cut in half vertically and covered with black cloth was the most effective. Bottles cut horizontally of with two sided vents were least effective in attracting the vector. *M. brunneum* was effective in controlling all larval stages in a dose dependant manner with highest mortality observed at 1x10⁸ conidia/ml. The first and second instars were more susceptible.

The above studies were complemented with human centred design studies, whereby, relevant stakeholders and consumers were consulted on the potential field application of the traps. Field experiments had been performed by installing the traps in houses. Interviews / group discussion of house-hold women had been done to get knowledge about various aspects regarding dengue fever and mosquitoes, fever symptoms, vector recognition, devices and cost of mosquito repellents / killing traps / dispensers, treatment cost, and physical and mental burden a family can feel in case of fever patient in the house.

264 **“If You Ain’t First, You’re Last”: A Spatial Approach to Pre-Hatch Larviciding**

Dan Bartlett, dbartlett@nwmadil.com, Emmanuel Arebanmhen, Julia LaValle, Patrick Irwin

As Mosquito Abatement Districts (MADs) and other agencies tasked with mosquito control are adjusting to new practices due to COVID-19, mainly the issue of hiring and retaining seasonal

staff, we wonder if our approach to ground-based, pre-hatch larviciding treatments is of use to our colleagues. Our applied research in summer 2022 used Geographic Information Systems (GIS) to help the Northwest MAD (Wheeling, IL, USA) define several appropriate study areas for a robust evaluation of pre-hatch larvicide efficacy in partnership with industry, seasonal employees, and interns in field operations, entomology, and GIS. Through uniform spatial analyses of study areas and the implementation of mobile GIS, we were able to target treatments and surveillance spatially. We also hope to gain insight from AMCA membership on the best practices for rearing larvae from treated sources in comparison to our methods. In addition to product evaluation, we observed benefits to survey practices for cataloging sources and received positive feedback from several residents directly adjacent to the study areas. Our thinking was to help alleviate the resource burdens of responding to “the water” or flooding events, instead target specific areas prior to these events, perhaps prior to the arrival of seasonal staff.

265 **Sublethal effects of Pyriproxyfen and altosid against *Aedes aegypti***

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Mosquitoes are amongst the anthropophagic insects because they feed on human blood and have medical importance to human as pest and vectors of some most distressing diseases. Mosquitoes borne disease currently represent greater health problems in tropical and subtropical climate and no part of world is immune to this risk. *Aedes* mosquitoes are best known vector of yellow fever and dengue fever. Different growth regulators are used for the control of mosquitoes. These effect the life span of different growth stages. So, the present study was carried out to evaluate the effect of insect growth regulator (pyriproxifen and altosid) on the life span of different growth stages. Three different dosage pattern of pyriproxifen and altosid Pro-G low dose, under dose, high dose will be used under lab conditions. Five serial concentrations (66, 6.6, 0.66, 0.066 and 0.0066) were used and the mortality data were taken after 24, 48 and 72 hr. Larvae were placed in 250 ml pyrex glass beaker with 200 ml distilled water and respective insecticide concentration. The present research concluded that pyriproxifen and altosid is an effective in controlling immature population. The altosid was found the best as compared to pyriproxifen in retarding larval population that increased larval duration and time to complete the life span. In this respect the fecundity of the *Aedes* population was also suppressed appreciably.

266 **Updates on the ongoing development of IDX (MosID/TickID), an imaging and identification device to support routine vector surveillance.**

Adam Goodwin, adam@vectech.io, Jewell Brey, Sanket Padmanabhan, Ghnana Madineni, Sameerah Talafha, Tristan Ford, Roy Faiman

Vector surveillance is critical to integrated vector management. However, identification is often time consuming and requires significant expertise. For mosquitoes, some vector control organizations have significant regional expertise in identification of the local species. However the task is time consuming and arduous, requiring experts to sift through the thousands of specimens captured by a fleet of traps multiple times a week. With funding constraints, many organizations delegate this task to seasonal workers, resulting in a high training burden and variable accuracy and speed. Furthermore, many organizations are beginning to expand operations to include tick surveillance. Tick identification requires its own specialized training, creating a barrier for many vector surveillance organizations who

aim to create or expand active tick surveillance programs. To help address these gaps, Vectech is developing IDX, a versatile tool for the imaging and identification of small arthropods, with an initial focus on critical vectors for disease such as mosquitoes and ticks. To date, development of tick species identification capabilities have achieved $98.5 \pm 0.3\%$ macro-averaged F1 score on IDX image datasets including 12 species, nine of which are commonly found in the US, including *Rhipicephalus sanguineus*, *Ixodes pacificus*, *Ix scapularis*, *Dermacentor variabilis*, *Haemaphysalis longicornis*, and *Amblyomma americanum*. Development of mosquito species identification has continued to include 23 species, achieving 94% macro-averaged F1 score including *Aedes aegypti*, *Ae albopictus*, *Anopheles albimanus*, *An gambiae* sl, *An stephensi* sl, and *Culex pipiens* sl. IDX identification algorithms are deep learning based, using convolutional neural networks, which require high data volumes. To this end, 12 IDX devices have been distributed to early partners who aid in gathering image data and algorithm assessment for both ticks and mosquitoes.

267 **Novel Tools for Increasing Mosquito Surveillance Efficiency: Using Machine Learning to Develop an Aedes Egg Counter App**

Kyndall Dye-Braumuller, kyndallb@email.sc.edu, Adam Frederiksen, Lawrence Miao, Jun Zhou, Bob Doran, Andre Da Costa Da Silva, Alisa Nelson, Huixuan Li, Matthew DeGennaro, Sarah Gunter, Melissa Nolan

In a typical mosquito surveillance and control operation, the bottleneck of mosquito identification and processing is almost always counting collected adults and or eggs. Further, when collecting eggs in the field, lighting and magnification are not always optimal to receive results in a timely matter—much less information on species. This project, as part of a larger *Aedes* ecology and breeding habitat NIH grant, aimed to simplify and expedite the egg counting and species identification portion of egg collections—from the field and from a laboratory colony. Through collaboration with the University of South Carolina’s Research Cyberinfrastructure program, a machine-learning-based mosquito egg counting algorithm was constructed and applied to count *Aedes* eggs on germination paper. Images were taken from multiple smartphones, different colored egg papers were used, various angles and magnification were tested, and a stand for phone stabilization was even printed via a 3D printer to standardize image capture. All images were counted by a laboratory technician to compare the app accuracy to human counting. We then developed a new Convolutional Neural Network (CNN) based algorithm to detect the eggs in these images. The algorithm can also detect clusters of eggs efficiently. Our algorithm outperforms many classical and deep-learning-based methods in both accuracy and computation efficiency, performing with 92% accuracy. Future directions include distinguishing *Aedes aegypti* and *Aedes albopictus* species through egg images and expanding the phone-based application to various geographies across the United States. The program is still being finalized; however, we anticipate this application to have wide-spread impacts on those in mosquito control and surveillance who need to identify and count mosquito eggs quickly for field collection and laboratory colony maintenance.

268 **An update on MosquitoMate’s autocidal approaches to control Aedes mosquitoes**

James Mains, jmains@mosquitomate.com, Stephen Dobson, Karen Dobson

Aedes aegypti and *Aedes albopictus* are both invasive species and public health concerns due to their aggressive day-biting behavior and ability to vector medically important pathogens

(e.g., Zika, dengue, chikungunya). Despite the heavy use of chemical pesticides to manage these species, they have colonized much of the U.S.A. and continue to expand their range. Multiple autocidal approaches have been proposed for the control of these species. Autocidal technologies are 'self delivering,' and there is no known insecticide resistance. These technologies use the 'mosquito against itself,' and include Wolbachia-based and auto-dissemination of insecticides. Similar to traditional Sterile Insect Technique, the Wolbachia approach is based upon repeated, inundative releases of incompatible male mosquitoes, with the goal of decreasing the number of viable eggs. The ADAM approach uses repeated, inundative releases of non-biting males as vehicles to deliver small doses of a potent insect juvenile hormone analogue (pyriproxyfen; 'PPF') into the small, cryptic containers in which *Ae. aegypti* and *Ae. albopictus* often breed. Males can deliver PPF to breeding sites either directly or indirectly, by cross contaminating females during copulation attempts. Presented here, are regulatory updates related to these technologies and results from recent open field trials used to examine for efficacy under field conditions.

Latin American Symposium I

269 **Reduction Of Enzymatic Activity Of Glutathione S-Transferase And Mixed Function Oxidases In Response To Insecticide Exposure And Denv-2 Viral Challenge In *Aedes Aegypti*.**

Alan Juache-Villagrana, alan.juache@gmail.com, Victoria Pando-Robles, Gustavo Ponce-Garcia, Beatriz Lopez-Monroy, Ildefonso Fernández-Salas, Adriana E. Flores

The enzymatic activity of glutathione S transferase (GST) and mixed function oxidases (MFO) regulates insecticide resistance and vector competence in *Aedes aegypti*. These enzymes' activity is affected by traits such as mosquitoes' age and feeding status. It is unknown how insecticide exposure and viral challenge could alter GST and MFO despite covariates such as age and feeding status. This work aims to determine the effects of DENV-2 viral challenge and permethrin exposure on the activity of GST and MFO regardless of age and feeding status.

We measured the activity of these enzymes in the thorax of ~30 females of *Ae. aegypti* (New Orleans Strain) using spectrophotometric techniques. Seven treatments were evaluated: 1) one d old, starved females, 2) 6 d old sugar-fed females, 3) blood-fed females, 4) oral DENV-2 challenged females, 5) permethrin pre-exposed and sugar-fed females, 6) permethrin pre-exposed and blood-fed females, 7) permethrin pre-exposed and oral DENV-2 challenged females. Three age groups of 6, 12, and 19 d old mosquitoes were used from treatment three to seven. A generalized linear model was used to determine the effect of permethrin exposure and viral challenge on the activity of GST and MFO.

Our results show that the activity of GST and MFO increases ($p < 0.0001$) proportionally with age and in the group of blood-fed individuals ($p < 0.0001$). However, when fixing the age and meal type, the marginal effect of permethrin exposure and viral challenge resulted in a reduction of GST and MFO activity ($p < 0.001$). Also, there is a statistically significant interaction between age and feeding, age and viral challenge, and feeding and viral challenge ($p < 0.001$). Our results show that insecticide exposure and viral challenge alter enzymatic activity in a reference strain of *Ae. aegypti* regardless of other biologically relevant variables such as age and meal type.

270 **The activity of a lipid synthesis inhibitor, spiromesifen, in *Aedes aegypti* (L.) (Diptera: Culicidae).**

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Spiromesifen is a systemic product belonging to the chemical group of spirocyclic tetronic acid derivatives, widely used against crop pests. This insecticide/miticide has become an important element in resistance management programs due to its mode of action and low toxicity against non-target organisms. It acts on lipid synthesis by inhibiting acetyl CoA carboxylase. An exploratory study was carried out to determine the biological effects of spiromesifen on *Aedes aegypti*. For this purpose, a field population (Guadalupe) with high frequency and intensity of resistance to temephos and the susceptible reference strain New Orleans were subjected to different concentrations of spiromesifen, a.i., to obtain the dose-response parameters: 0.01 to 15 mg/L (New Orleans) and 0.1 to 25 mg/L (Guadalupe) in early 4th instar larvae according to the protocol of WHO (1981). Mortality was monitored daily until the emergence of adults, and the inhibition of emergence (IE) was calculated. The results were subjected to probit analysis (PoloPlus 2.0) to determine LC₅₀, whose values were 1.211 mg/L (95% CI: 0.4536-0.0699; slope: 0.5684; X₂: 58.78) and 1.807 mg/L IC 95%: 0.6556-0.3089; slope: 0.9579; X₂: 25.48) for New Orleans strain and Guadalupe population, respectively. Subsequently, early 4th instar larvae of the susceptible strain and the field population were exposed to their respective LC₅₀ in separated groups for 24, 48, and 72h to analyze the effect on the biochemical components (proteins, carbohydrates, and lipids), biomarkers of oxidative stress (malonaldehyde and catalase) as well as evaluation of body weight and volume. The results show that the product reduced the larvae's growth in addition to inducing morphological aberrations and affecting the body volume and the content of carbohydrates, lipids, and proteins with a significant effect on exposure at 48h. Oxidative stress biomarkers and lipid content indicate that spiromesifen influenced metabolism, confirming its mode of action in lipid inhibition.

271 **Gene flow and kdr mutations in populations of *Triatoma dimidiata* (Latreille, 1811) (Hemiptera: Reduviidae, Triatominae) from Mexico.**

Jesus A. Davila-Barboza, jdavilab@uanl.edu.mx, Mario Saucedo-Montalvo, Susana Favela-Lara, Beatriz López-Monroy, Gustavo Ponce-Garcia, Adriana E. Flores-Suarez

Gene flow is responsible for the exchange of genes between populations, which promotes the acquisition of alleles that can influence in biological aspects and resilience of *T. dimidiata*, one of the most relevant species in the transmission of the Chagas disease in Mexico due to the intradomiciliary infestation that it exhibits in rural dwellings, however, recent studies have registered a higher incidence in urban areas.

Studies have been carried out on genetic fluctuation in triatomines using molecular markers such as. microsatellites and mitochondrial sequences, however, studies of this type in Mexico for *T. dimidiata* are scarce. On the other hand, it is unknown how the genetic variability of populations can influence the presence of kdr mutations that confer resistance to pyrethroid insecticides.

In this work, the mitochondrial markers ND4 and CytB were used to analyze the components of genetic variation and fixation index within and between the populations collected in the states of Yucatan, Veracruz, and Oaxaca, in addition to detecting the presence of *kdr* mutations in the para gene of these populations.

The use of such molecular tools made it possible to detect a genetic differentiation between the populations, identifying the various haplogroups present, as well as registering the presence of the *kdr* L1014F mutation associated with resistance to pyrethroids.

272 **miRNomics universe of *Aedes aegypti* (L) (DIPTERA: CULICIDAE)**

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The microRNAs (miRNAs) are small single-stranded endogenous RNAs with a length between 21 and 25 nucleotides and these regulate the translation of proteins by hybridization with messenger RNAs. To date, its existence and physiological role have been reported in organisms of all kingdoms. The microRNAs participate by performing multiple and varied functions. To date, it has been shown that miRNAs in mosquitoes play important roles in various biological processes such as blood digestion, oviposition, sexual differentiation, resistance to insecticides and infection with pathogens. At this moment, microRNAs from 33 insect species have been reported in the miRBase database, with a total of 3,857 microRNAs. In this work we describe the battery of microRNAs with exclusive expression of diverse biological approaches that had at least 10 reads and 5 folds of presence. Now we know microRNAs with an exclusive expression in males (10), females (7), adults (45), eggs (13), females fed and sacrificed at 24 (11), 48 and 72 hours. In the present study we report for the first time the mirnomic fingerprint of diverse biological approaches [life stages (eggs and adults), sex (males and females) and the postprandial chronology (0, 24, 48 and 72 hours after feeding)] of *Ae. aegypti*. The differential expression pattern of the miRNAs specific and shared in stages of life plays a critical role in the physiology of *Ae. aegypti*. A better understanding of the functions of these miRNAs will have a great implication for the effective control of the population of vectors and, therefore, to interrupt the transmission of diseases caused by pathogens such as Dengue, Zika, Chikungunya, Mayaro and Sepik viruses.

273 **SPATIAL AND TEMPORAL DYNAMIC OF KDR MUTATIONS IN *Aedes argypti* AND ITS ASSOCIATION WITH SOCIODEMOGRAPHIC AND ENVIRONMENTAL CHARACTERISTICS**

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Aedes aegypti (Linnaeus, 1762) is the most important arbovirus vector with wide distribution worldwide. In our study, we evaluated the spatio-temporal patterns of *kdr* mutation frequencies in relation to sociodemographic and environmental variables. The genotypic and allelic frequencies of *kdr* mutations in Nuevo Leon, Veracruz and Yucatan, Mexico were monitored in 2020 and 2021, as well as the use of insecticides. We report 23 of 27 possible haplotypes, being the triple mutated (L410/L410-I1016/I1016-C1534/C1534) the most frequent, followed by the homozygous mutated haplotype in 1016 and 1534 in combination with the heterozygous genotype in 410. (V410/L410-

I1016/I1016-C1534/C1534). According to the results, it was determined the influence of temperature, rainfall, number of inhabitants, type of locality and the presence of other kdr mutations on the frequency of mutated alleles. It was observed for samples recollected in 2020 that mutated allele C1534 frequency influenced frequency of mutated alleles I1016 and L410, while the frequency of the mutated allele L410 is influenced by the number of inhabitants in localities. In samples of 2021 we report that frequency of the mutated allele C1534 was influenced by the frequency of the allele L410, while the frequency of L410 was influenced by temperature and the average annual rainfall of the locality. In addition to this, spatial prediction IDW maps were made using the frequency of mutations obtained for both years, which were contrasted with the history of insecticide application in the collection sites, as well as the percentage of mortality obtained from bioassays from the CDC with diagnostic dose of deltamethrin.

274 **Socioeconomic and Environmental Factors associated with Risk of Dengue Fever Incidence in Guatemala (2017-2018)**

Kasem Salim, ksalim4@ucmerced.edu, Andrea Joyce

Dengue Fever outbreaks in Guatemala are occurring at increased rates. This study examined socioeconomic and environmental factors associated with Dengue in Guatemala. Socioeconomic factors included population density, literacy, internet use, Mayan speakers, economic activity, and attending school. Environmental factors included elevation, temperature, and precipitation. Zero-inflated negative binomial regressions found population density, internet use, attending school, Mayan speakers, and temperature were significantly associated with Dengue. Environmental and socioeconomic factors both relate to Dengue incidence in Guatemala.

275 **Population genetic structure of Aedes aegypti in El Salvador and Honduras**

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Aedes aegypti is associated with dengue, yellow fever, chikungunya and Zika viruses. This vector is widespread in tropical and subtropical areas, and can also occur in temperate areas at higher latitudes. This study examines the population genetic structure of *Aedes aegypti* in El Salvador and Honduras. Larvae were collected in 12 regions (municipalities) in El Salvador, and from 6 in Honduras; larvae were raised into adults and identified. DNA was extracted, and the mitochondrial DNA cytochrome oxidase I (mtDNA COI) barcode was sequenced and compared among individuals from all the populations. Data were used to determine genetic distance between populations, haplotype diversity, produce a haplotype tree, and a phylogenetic tree. Results will be discussed and considered in light of SIT, IIT, and other novel mosquito control technologies.

276 **Technological Innovation in Mosquito Monitoring (Species, Genus and Age)**

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Technological Innovation in Mosquito Monitoring (Species, Genus and Age)

Process automation, generate surveillance data in real time, reduction of logistics costs, human resources, time optimization and correct prioritization of hot spots.

IoT sensor application for mosquito monitoring:

Remote and automatic classification of mosquitoes: counts, genus, species, sex and age.

Provides near real time information.

Savings of 80% in field operations costs

Embedded in existing applications based on satellite and citizen science data modelling, already being used by health agencies: VECMAP, Mosquito Alert

Lab results so far- Data analysis using machine learning techniques allow us to separate the different variables tested (species, sex and age) with a range accuracy from 61,3% to 99% .

Continuous studies with different mosquito species

Species list (target species)

Aedes aegypti

Aedes albopictus

Aedes caspius

Aedes vexans

Anopheles atroparvus

Culex laticinctus

Culex pipiens

Culex theileri

Culex hortensis

Culiseta longiareolata

Chironomidae

Variables list

Species

Sex

Age (2-14 days)

Temperature (18-28°C)

Size (large, small)

Parity (nulliparous, parous, blood fed, gravid)

Nutritional status (starved, sugar fed, blood fed)

Value for money: Our sensor provides real-time data, or a daily report in the worst case scenario. To provide daily data using SoA traps, users would have to increase their field operation costs 15-20 times.

Scalability and Flexibility: The sensor is designed to be modular in field deployment, and is highly robust as proven with field trials running since 2018 (at least 5 year life time). It can be easily adapted to different types of traps: adult traps, gravid traps and ovitraps.

Power needs: Sensor consumes less power than the fan of commercial adult traps. In the case of adult traps, the sensor can be powered the same way as the trap itself: power grid or batteries.

Communications: Mobile networks (2G, 3G, 4G, 5G...) with world coverage IoT SIM. Other technologies can be used like WiFi, Bluetooth and LPWAN.

Easy to use: The sensor is easily attached to commercial traps. As soon as it is powered, it will automatically initiate itself, run diagnostics, and start collecting and transmitting data. The sensor does not even have a turn on-off button.

IoT standards: OGC-SWE- (Open Geospatial Consortium - Sensor Web Enablement), IEE1451- (Smart Transducer Interface for Sensors and Actuators).

Mosquito classification: The sensor is trained in lab trials with known species of mosquitoes. This enables creating Machine Learning algorithms for those species. Nowadays the sensor is trained for *Culex pipiens*, *Aedes aegypti* and *Aedes albopictus*.

Accuracy: With the algorithms, we make the identification of only flying insects (we reject any other object that is not a flying insect, like suspended dust particles, pollen, rain, leaves, etc.). The sensor can be used to detect Genus, Species and Sex in areas colonized with mosquitoes for which the sensor has been trained. If the sensor is placed in an area colonized by "unknown" species, the sensor is still capable of classifying Genus and Sex.

277 **Insecticide susceptibility and resistance mechanisms in *Aedes aegypti* from urban and rural areas of Venezuela**

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The massive use of insecticides in public health has exerted selective pressure resulting in the development of resistance in *Aedes aegypti* to different insecticides in Venezuela even though since 2010 the only insecticides available for vector control were the organophosphates fenitrothion and temephos which were focally applied. To determine the state of insecticide susceptibility and to identify the possible biochemical and molecular mechanisms involved in resistance of *Ae. aegypti* in Venezuela, CDC bottle bioassays were conducted on *Ae. aegypti* collected between November 2019 and January 2020 in two urban and one rural locality. Insecticide resistance mechanisms were studied based on levels of detoxifying enzymes, insensitivity target site for organophosphates and allele-specific RT-PCR to detect mutations V410L, F1534C, and V1016I. All strains showed significantly higher activity of the enzymes acetylcholinesterase, mixed function oxidases, and glutathione-S-transferases in comparison with the susceptible strain. The *kdr* mutations V410L, F1534C, and V1016I were detected in all strains, with F1534C at higher frequencies in all strains. Insecticide resistance persists in *Ae. aegypti* strains even in the relative absence of insecticide application. Successful vector-borne disease control

will require major investment in vector control infrastructure and in effective, and sustainable interventions.

Transforming surveillance and control effort using GIS/RS Symposium

278 (Re)using published georeferences with Biodiversity Enhanced Location Services (BELS)

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The research potential for data associated with museum specimens of insect vectors has changed dramatically due to the production of tools and community coordination around digitization and mobilization. However, many specimen records still only have textual descriptions of geographic locations where collecting occurred. Georeferencing - the conversion of these textual descriptions to mappable coordinates with associated uncertainties - still remains a significant bottleneck to the ready use of these data. The majority of data records published to aggregators such as iDigBio and GBIF still lack proper georeferences. The standards and best practices for georeferencing are mature. Tools such as Geolocate support generation of georeferencing metadata reflecting those best practices, e.g. valid coordinates, datum and information about spatial uncertainty. Of all the means to speed up georeferencing, one that potentially requires the least amount of extra work is to find and use a location record that is already properly georeferenced. Enter BELS: Biodiversity Enhanced Locality Services, built around a database of previously georeferenced locations found in GBIF, iDigBio, and the VertNet collaborative georeferencing projects. Using this gazetteer, we determine how many non-georeferenced locations may have georeferences retrieved from previous efforts. In this process, we explore these databases to determine the number and quality of the data available. Finally, we explore use-case datasets to examine the value of BELS for the community. We also discuss the integration of the gazetteer in commonly used tools such as GeoLocate and Symbiota. Integration with existing toolchains assures maximal uptake by the community.

279 Employing Spatiotemporal Data for Disease and Non-Battle Injury Risk Reduction in Deployed Environments

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Disease and Non-battle Injury (DNBI) has long been the greatest cause of mortality and morbidity for military forces in both Garrison and deployed settings, far exceeding battle-related injury. To maintain optimal readiness, reduction of DNBI is the primary responsibility of Preventive Medicine (PM) officers in the Army. One focus of PM officers is the control of vector-borne disease. To this end, the traditional methods of integrated pest management (IPM) – biological, cultural, mechanical/physical, and chemical controls - are frequently employed. However, access to Geographic Information Systems (GIS) and Remote Sensing (RS) data has allowed PM officers to improve targeting of vectors for application of IPM controls while further reducing personnel health risk by working with commanders to select forward operating bases in locations identified as lower risk for disease transmission. This presentation will discuss the use of GIS, RS data, and ecological niche modeling of vector-borne disease by U.S. military forces to improve operational readiness through DNBI risk reduction in deployed environments.

Julie Allen, jallen23@unr.edu

Identifying the factors that affect parasitic arthropod distributions is important for understanding disease transmission, and parasite richness/diversity. Ticks (Arachnida: Ixodida) are obligate ectoparasites that require hosts to obtain blood meals, and often vector pathogens. Because of their vector potential, it is necessary to understand tick distributions and the variables that influence their presence. This study focused on determining the factors that affect tick distributions on birds sampled across different elevations and habitats in Colombia. The model took into account both habitat variables and avian traits to determine the relative importance of these variables in predicting tick presence and distributions. We sampled >800 birds for ticks across environmental gradients in Colombia. We focused on 14 microsystem variables related to the avian host (e.g. diet, canopy level), and 27 macrosystem variables of the host's environment (e.g. tree cover, precipitation). Using a binomial regression model we determined the top predicting factors in each of these models and created a combined model with each of the top predicting factors to determine which factors are most important in predicting tick distributions host or habitat.

Global Mosquito Observations Dashboard (GMOD): integrating citizen science platforms to enable next-generation surveillance of invasive and vector mosquitoes

Ryan Carney, ryanmcarney@gmail.com, Connor Mapes, Johnny Uelmen, Russanne Low, Alex Long, Anne Bowser, David Durieux, Karlene Rivera, Berj Dekramanjan, Frederic Bartumeus, Daniel Guerrero, Carrie Seltzer, 12:00:00 AM, 12:00:00 AM, John Palmer,

Mosquito-borne diseases continue to ravage humankind with >700 million infections and nearly one million deaths every year. Yet only a small percentage of the >3500 mosquito species transmit diseases, necessitating both extensive surveillance and precise identification. Unfortunately, such efforts are costly, time-consuming, and require entomological expertise. As envisioned by the Global Mosquito Alert Consortium, citizen science can provide a scalable solution. However, disparate data standards across existing platforms have thus far precluded truly global integration. Here, utilizing Open Geospatial Consortium standards, we harmonized four data streams from three established mobile apps—Mosquito Alert, iNaturalist, and GLOBE Observer's Mosquito Habitat Mapper and Land Cover—to facilitate interoperability and utility for mosquito control personnel, researchers, and policymakers. This GIS mapping platform, the Global Mosquito Observations Dashboard (GMOD), is freely accessible at www.mosquitodashboard.org for visualizing and downloading data in various tabular and geospatial formats. We also launched coordinated media campaigns that generated unprecedented numbers and types of observations, including successfully capturing the first images of targeted invasive and vector species such as *Aedes scapularis* and *Aedes vittatus*. For mosquito control organizations, such citizen science efforts can contribute valuable surveillance data to complement traditional trapping methods and to validate habitat models. Additionally, we leveraged pooled image data along with imagery generated in collaboration with the CDC, to develop a toolset of artificial intelligence (AI) algorithms for deployment in taxonomic and anatomical identification. The beta version of our AI tools for analyzing photos of larval and adult mosquitoes are freely available at www.mosquitoID.org, primarily targeting the urban malaria vector *Anopheles stephensi* that has recently invaded Africa. Ultimately, by harnessing the combined powers of citizen science and artificial intelligence, we establish

a next-generation surveillance framework to serve as a united front to combat the ongoing threat of mosquito-borne diseases worldwide. [publication: bit.ly/3RyztIo]

282 Leveraging GIS, remote sensing, and vector surveillance data to predict joint distributions of arbovirus mosquito vectors

Amely Bauer, amelybauer@ufl.edu, Mohamed Sallam, Lindsay Campbell

Geographic information systems (GIS) and remote sensing technologies offer new opportunities to investigate environmental correlations with mosquito vector species. Advances in modeling approaches combined with routinely collected trap data provide the basis to maximize mosquito control surveillance to predict distributions of individual and combinations of mosquito vector species. One approach to predicting the distribution of multiple mosquito vectors across different landscapes is the use of a joint species distribution modeling framework (jSDM). Rather than predicting a single vector species, this method generates model outputs that allow predictions of multiple species and their traits, including vector competency. Here, we used a jSDM approach to investigate correlations between mosquito community composition, community-weighted mean traits (CWMT), and LULC in Manatee County, Florida to identify areas predicted to have high proportions of West Nile virus vectors. Species presence/absence across 60 trap sites sampled in 2016, 2017, 2019, and 2020, served as response variables; percent land cover for developed, cropland, herbaceous wetland, and woody wetland within 5 km of trap sites served as environmental variables. Community weighted mean values were calculated from a binary matrix of WNV vector competency to predict proportions of WNV vectors across the study area. Results indicated that proportions of variance explained by percent land cover for individual species coincided with known habitat associations for most species. Maps visualizing spatial predictions of CWMT predicted mosquito communities with the highest proportions of WNV vector competent species in urbanized areas. Joint species distribution models that leverage GIS and remote sensing data provide a powerful tool that can help optimize targeted surveillance and control efforts and have the potential to provide insights that may help improve prevention and management of mosquito-borne disease.

283 High resolution ensemble species distribution models for mosquito vectors (Diptera:Culicidae)

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In the absence of entomological information, tools for predicting mosquito vector species presence can help evaluate the entomological risk of vector-borne disease transmission. Here, we illustrate how species distribution models (SDM) could quantify potential dominant vector species presence in several settings. We fitted a 250 m resolution ensemble SDM for *Anopheles albimanus* Wiedemann, a dominant malaria vector, and a 30 m resolution SDM for *Aedes aegypti* L, a dominant dengue vector. Each ensemble SDM included predictions based on several algorithms. SDM covariates included environmental variables that were selected based on their importance from a larger set of layers that included remotely and spatially interpolated locally measured variables for the land surface of Costa Rica. Goodness of fit for each ensemble SDMs was very high, with a minimum AUC of 0.79. We used the resulting ensemble SDMs to evaluate differences in habitat suitability (HS) between commercial plantations and surrounding landscapes, finding a higher HS in pineapple and oil palm plantations, suggestive of *An. albimanus* and *Ae. aegypti* presence, than in surrounding landscapes. The

An. albimanus ensemble SDM suggested a low HS for An. albimanus at the presumed epicenter of malaria transmission during 2018-2019 in Costa Rica, yet this vector was likely present at two villages also affected by the epidemic. Our results illustrate how ensemble SDMs in malaria elimination settings, and more generally in vector-borne disease transmission settings, can provide information that could help to improve vector surveillance and control .

284 **Web GIS – How A Single Source of Information Benefits Your Organization**

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Leveraging the Esri ArcGIS Online platform as the single source of information for your organization has many benefits. Coupled with the Frontier FieldSeeker GIS mosquito control software the user is provided with simple, intuitive tools for finding, collecting, and reporting data related to control activities. Data from other sources (ATV / truck spraying systems, aerial data from drones, planes, or helicopters systems, asset management systems, public notification systems, etc.) can also be stored in or integrated within the GIS. Field and office users benefit from seeing a common up-to-date picture of their operations. The more data an organization can share, the better able it is to make plans to move forward with its goals while minimizing time spent reconciling data sets for required reporting.

We will also overview recent products updates to our FieldSeeker Core software (workflows for Larviciding with storm drain treatments, Surveillance, and Service Request) and our FieldSeeker ULV Adulticiding system.

285 **Co-occurrence probabilities between mosquito vectors of West Nile and Eastern equine encephalitis viruses using Markov Random Fields (MRFcov).**

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Mosquito vectors of eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) in the US reside within broad multi-species assemblages that vary in spatial and temporal composition, relative abundances, and vector competence. These variations impact the risk of pathogen transmission and the operational management of these species by local public health vector control districts. However, most models of mosquito vector dynamics focus on single species and do not account for co-occurrence probabilities between mosquito species pairs across environmental gradients. In this investigation, we use for the first time conditional Markov Random Fields (CRF) to evaluate spatial co-occurrence patterns between host-seeking mosquito vectors of EEEV and WNV around sampling sites in Manatee County, FL. Specifically, we aimed to 1) quantify correlations between mosquito vector species and other mosquito species, 2) quantify correlations between mosquito vectors and landscape and climate variables, and 3) investigate whether the strength of correlations between species pairs are conditional on landscape or climate variables. We hypothesized that either mosquito species pairs co-occur in patterns driven by the landscape and/or climate variables, or these vector species pairs are unconditionally dependent on each other regardless of the environmental variables. Results indicated that landscape and bioclimatic covariates did not substantially improve the overall model performance and that the log abundances of the majority of WNV and EEEV vector species were positively dependent on other vector and non-vector mosquito species, unconditionally. Only five individual mosquito vectors were weakly dependent on environmental variables with one exception, *Culiseta melanura*, the primary vector for EEEV, which showed a strong correlation with woody wetland, precipitation seasonality, and

average temperature of driest quarter. Our analyses showed that majority of the studied mosquito species' abundance and distribution are insignificantly better predicted by the biotic correlations than environmental variables. Additionally, these mosquito vector species may be habitat

286 **Methods helping to undercover hidden diversity**

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The chewing lice genus *Myrsidea* is known for its hyper-diversity and host-specificity, with more than 80% of its species parasitizing only on one bird host species. *Myrsidea* are permanent ectoparasites of birds, meaning, they live the whole life on their hosts. They often affect native species of birds in our wildlife and can cause their hosts distress or secondary infections. This could further result in death of the bird and cause imbalance in the food chain. Interestingly, birds are the most diverse group of land vertebrates with more than 10,000 species known, from many of which could potentially harbor *Myrsidea*. While some of the regions are relatively well studied (e.g. Neotropic and Oriental region), it is still only "a tip of the iceberg" as most of the *Myrsidea* species are expected yet to be described. In the current study we evaluated the spatial distribution of *Myrsidea* over time on global scale using GIS techniques. We characterized the landscape and climate variables at areas under risk of heavy infestations for conservation purposes and decision making to control these pests. Spatial discrepancies in distribution of this pest were discussed in response to biophysical covariates. Additionally, knowledge gaps and future directions were identified in the light of our spatial analysis.

Disease/Vector Studies II

288 **Detection of Dengue Virus Serotype-1 in *Aedes aegypti* mosquitoes collected during an outbreak in Araraquara, Brazil using Integrated *Aedes aegypti* Monitoring (MI-Aedes) technology**

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Araraquara is a medium-sized municipality in São Paulo state, Brazil, that reported 23,847 cases of dengue in 2019. In 2022, a similar scenario was experienced, with approximately 16,395 cases and 17 deaths. The purpose of the study was to describe the infection rate in mosquitoes caught in traps distributed in the urban areas of the city. **METHODS:** From epidemiological week (EW) 02 to 22 of 2022, 909 Mosquitrap installed in the municipality were weekly inspected. *Aedes aegypti* (males and females) were collected and analysed to examine the presence of Dengue Virus. Confirmed human cases were obtained through a database made available by the municipality. **RESULTS:** During the 20-week study period, the MFAI (Mean Female *Aedes* Index) ranged from 0.22 (EW 6) to 1.04 (EW 18), and 2611 dengue human cases were confirmed. The highest number of cases occurred from EW 09 to 11, and a second peak occurred from EW 20 to 22. 5233 mosquito samples were collected and 241 samples were positive for DENV, showing an infection rate of 4.36%. EW 17 and 18 had the highest infection rates, with positivity rates above 8%, and EW 12, 15, 16, and 19 also presented high positivity rates, showing percentages above 7%. EW 3, 5, and 6 yielded no positive results. **DISCUSSION:** The recurrence of a DENV1 epidemic reveals the seasonal profile of the disease in Brazilian endemic cities, such as Araraquara. Factors like the high density of *Aedes* and its distribution in the municipality most likely

contributed to the high number of human cases during the first half of 2022. The increase in cases and positivity in mosquitoes was not limited to a single area, demonstrating the virus's ability to spread rapidly throughout the city's urban areas.

289 **ASSESSMENT OF POSITIVITY RATE OF AEDES ALBOPICTUS AND AEDES AEGYPTI IN WESTERN PROVINCE, SRI LANKA**

M. M. S. M. B. Marasinghe, smbmara@gmail.com, A. P. S. Perera

In Sri Lanka, it is recognized that the primary and the secondary vectors for dengue transmission are *Aedes aegypti* and *Aedes albopictus* respectively which are invasive mosquitoes. All the districts are potentially suitable for the survival and establishment of both vectors. A desk review was conducted to analyze the vector positivity proportion in Western Province including all three administrative districts from 2018 to 2021 to establish the receptivity in the area to assist vector control interventions.

Vector positivity data were collected from Monthly house to house vector immature stages surveillances carried out by National Dengue Control Unit and local health sectors covering all the potential and existing breeding places in the study area to collect larval and pupal stages of the two vectors. The number of positive containers for any immature stage was recorded and identified to species level.

During the entomological surveillances conducted the *Ae. aegypti* positivity rate was gradually increased from 31% to 42% through the study period in Colombo district while in Gampaha and Kaluthara districts the same rate was marginally fluctuated around 15% and 3% respectively.

The increase trend of the *Ae. aegypti* proportion was vividly seen in Colombo district which is undoubtedly the most urbanized area of all with higher population density and land use. However, in Gampaha and Kaluthara districts which are with less population density, the increments of *Ae. aegypti* proportion were occurred moderately with minor fluctuation during all four years. Due to the continuous heavy urbanization rate *Ae. aegypti* is gradually replacing the secondary vector, *Ae. albopictus* in Colombo district when compared the vector positivity data with the other two counterpart districts. The vector control interventions should be adjusted accordingly while identifying the different vector bionomics possessed by the two vectors.

Key words: *Aedes aegypti*, *Aedes albopictus*, positivity, dengue, surveillance

290 **New distributional records and ecological notes of phlebotomine sandflies in northeastern Mexico**

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Phlebotominae sand flies are the vectors of several species of *Leishmania*, which are the etiological agents of leishmaniasis. In Mexico, the southeastern region is endemic to this disease and therefore many studies have been focused in that area. However, cases of leishmaniasis also occur in other Mexican states such as Coahuila, Nuevo León and Tamaulipas located in the northeast region bordering the USA. In Nuevo León, cases of cutaneous leishmaniasis have been reported intermittently through the years. Few records of sand fly species exist for this state and many municipalities remain unsampled despite the potential importance of the disease in certain regions. The main objective of this

work was to undertake sand fly catches in the municipality of General Zuazua, Nuevo León, which has had a rapid urbanization process in the recent years. A location was selected to conduct catches of sand fly species using CDC light traps in the period of 6th August through 26th September 2020. Nine samplings were carried out finding 137 specimens belonging to four genera and five species. The most predominant species was *Lutzomyia diabolica* that represented 90.51%. It was found that sand fly species had a peak at sampling 5 (34.31%) and that abundances between samplings were significantly different. Trap location was also found to be significant different between sites. Using Taylor's power law, it was found that spatial distribution was aggregated. None of the measured environmental variables such as air temperature, soil temperature, relative humidity, dew point or wind speed had a significant relationship to sand fly abundances. All five sand fly species found in this study are new records to the municipality of General Zuazua, Nuevo León being these *Dampfomyia anthophora*, *Micropygomyia apache*, *Micropygomyia californica*, *Psathyromyia texana* and *Lutzomyia diabolica*. *Micropygomyia californica* is recorded for the first time in México.

291 **Vector Surveillance and Control Response to a Japanese Encephalitis Outbreak in Humans and Commercial Pigs in Queensland, Australia.**

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Japanese encephalitis virus (JEV) was first detected in Australia in 1995 in the Torres Strait and in 1998 in the Cape York peninsula in northern Queensland. JEV detections were limited to genotype II in northern Queensland and Torres Straits until February 2022, when unexpectedly the virus, genotype IV, was detected in stillborn and mummified piglets in four states across south-eastern Australia, including Queensland. This was a rapid and monumental expansion of the known range of this virus.

Japanese encephalitis is a nationally notified disease in both humans and animals, and a category 1 emergency animal disease (EAD) in Australia. On 28 February 2022 the Consultative Committee for Emergency Animal Diseases met. On 4 March 2022, JEV was declared a Communicable Disease Incident of National Significance. As of September 2022, there have been 30 confirmed human cases, 10 probable or suspected cases, and 6 human deaths across Australia. In Queensland, there have been 2 confirmed cases, 2 probable or suspected cases, and one death attributed to JEV.

JEV infection has been confirmed at over 80 commercial piggeries across the four affected states. Businesses representing about 60% of the Australian pork industry have been impacted by the 2022 JEV outbreak, suffering substantial financial losses with preliminary estimates suggesting that the Australian fresh pork supply could fall between 9% and 18% nationally during the period from August to November 2022.

This presentation will describe the current knowledge about the vector ecology of JEV as it relates to Queensland, Australia, including known vector and reservoir host species, as well as outlining the national and state vector surveillance and control plans, and particular challenges controlling mosquito vectors within an animal and food production context.

292 **Spatio-temporal Variations and Intraspecific Morphological and Morphometric Variabilities of *Culex* larvae during the Cold Dry Season in Khartoum Sudan**

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A cross sectional survey using larval collection stander techniques was conducted during the cold dry season (October 2018 - February 2019) in Khartoum State to clarify effect of A/biotic factors on Culex population structure density and diversity. Larvae were obtained from 17 sites encompassing six different habitat types. The culicinae comprising 93.86% of the total collected larvae; the rest were Anophelinae 6.14%. Broken pipes were the most abundant habitat type (33.2%) followed by drain canal (25.7%), water pool (23.9%), irrigation canal (12.8%), water containers (3.1%) and others (1.3%); drain canals were the most reproductive one (174 larvae/dip). Alga, plant and other invertebrates high prevalence in breeding site the high densities of Culex larvae. Shadow and deep site positively increased productivity of Culex larvae. Morphological identification revealed that Cx .quinquefasciatus was the dominant species (69.1%) followed by morphologically similar Cx.univittatus (19.1%) and Cx.antenntus (11.7%). However, Cx. Quinquefasciatus showed intraspecific morphological differences.

293 **The Patterns of West Nile Mosquito Infection are Changing in Iberia Parish, Louisiana**

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Over the last 17 years, the numbers of annual WNV-positive pools generated by mosquito trapping in Iberia Parish, Louisiana have fluctuated greatly. Since 2010, they have generally remained low, less than 5 positive pools/yr. 2012 was a big outbreak year, when the parish generated 166 WNV-positive pools. 51 of those pools came from the city of New Iberia, with another 20 from the heavily industrialized northwestern part of the parish. 96% of the pools consisted of Culex quinquefasciatus. In 2013 and 2014, another 20 WNV-positive pools were obtained, all but 5 of them either from the city or from the northwestern corner of the parish. Beginning in 2018, the pattern changed. That year, 25 positive pools were obtained, only 2 of which came from the city and 2 from the northwestern sector. Most came from rural areas in the eastern part of the parish. In 2022, as of this writing, 13 positive pools have been obtained. All but 1 have been from rural areas, 8 from the eastern part of the parish. 4 pools have consisted of species other than Cx. quinquefasciatus, including Cx. erraticus, Cx. salinarius, and Anopheles quadrimaculatus. Changes in the distribution of West Nile in our mosquito population are possibly due to changes in its distribution in the bird population, with more birds from east of the parish bringing the virus into the area.

294 **Evaluation of Co-Diagnostics Vector Smart™ North American Mosquito East and West Multiplex assays and CoDx Box™ Thermal Cycler platform**

Kristy Burkhalter, ktb3@cdc.gov

Co-Diagnostics Vector Smart™ North American Mosquito (NAM) assays are RT-PCR multiplex tests marketed to mosquito control districts for the simultaneous detection of West Nile virus, St Louis encephalitis virus, and either Eastern equine encephalitis virus or Western equine encephalitis virus (NAM-e or NAM-w assay, respectively) in mosquito pools. We conducted an independent laboratory evaluation of these assays on a standard real time RT-PCR instrument and on the vendor's CoDx Box™ Thermal Cycler platform. Validation was performed on a variety of samples, including laboratory-generated virus-spiked mosquito pools and field-collected mosquito pools. Mosquito pools processed in the Rapid Analyte Measurement platform (RAMP™) assay buffer were also tested to validate the NAM assay's performance as a confirmation assay for that sample type. Sensitivity and specificity characteristics of the assay will be presented, as well as its technical, reagent, and equipment requirements.

295 **Development of an all-in-one metabarcoding approach to mosquito and arbovirus surveillance**

Brian Johnson, brian.johnson@qimrberghofer.edu.au, Melissa Graham, Nisa Suraj Nath, Elina Panahi, Gregor Devine

Next-generation sequencing (NGS) benefits to entomological and disease surveillance have been well-proven, yet the two approaches are rarely combined. Here, we describe the development and application of a metabarcoding approach to vector and arbovirus surveillance, including the identification of vertebrate hosts, to a large sample of light trap collections from Southeast Queensland, Australia. Virus surveillance was performed using a combination of pan-Alphavirus, pan-Flavivirus, and Ross River virus (RRV) specific primer sets. The resulting amplicons were combined and indexed with those targeting invertebrates (COI and ITS2) and vertebrate blood-meal hosts (CytB) prior to sequencing to reduce library preparation time and expense. DNA sequencing confirmed the presence of all morphologically identified mosquito species as well as commonly misidentified species and difficult to differentiate biting midge species (Culicoides). The general agreement between the proportion of NGS reads and individual species abundance allows users to reliably infer the overall composition of bulk light trap collections without morphological identification. Importantly, identification of a variety of vertebrate blood-meal hosts was observed, including humans and a variety of known reservoir host species for RRV and other endemic diseases, in the absence of visibly blood-fed females. Lastly, a diversity of insect-specific and medically important viruses, including RRV, was detected. Overall, metabarcoding shows great promise as an efficient vector and arbovirus surveillance tool and means of elucidating spatially and temporally variable vector-host relationships.

Education/Genetics

296 **Full Steam Ahead: Destination Education**

Cindy Mulla, cindy@pcbeachmosquito.com

Beach Mosquito Control District continues to build up STEAM (Science, Technology, Engineering, Art, and Mathematics) programs for local area schools. These unique laboratory classes teach mosquito surveillance techniques and identification. The goal of the curriculum is to sharpen the individual student's skills to improve scientific data recording, reinforce the scientific method and open young scientific-thinking minds.

297 **Visual Accessibility of Media in Mosquito Control Public Education**

Emily Evans, emyvans@gmail.com

Public education has long been an essential facet of Integrated Mosquito Management. The purpose of public education is to assist the community in understanding its role and responsibility towards local mosquito abatement efforts. Within the near 100-year documented history of American mosquito control, practically every form of visual media has been incorporated at this point. In the best interest of furthering such efforts, any media created by mosquito control should strive to garner the most community engagement. One, often unprioritized method to increase community engagement

from public education is to consider the visual accessibility of its media. Therefore, this presentation will highlight challenges faced by media consumers and ways to mitigate them.

298 **Creating a School Education Program as a Non-Educator**

Andrea McKinney, amckinney@cmcd.org

Public outreach and education are an important component for an effective Integrated Mosquito Management program. Teaching children effective mosquito prevention can instill habits and behaviors to last them a lifetime. In 2018, the Collier Mosquito Control District added a Public Outreach Specialist to design and implement a school-based mosquito education program from the ground up. Without an education degree or formal teaching background, but brimming with extensive knowledge about mosquitoes, where would she start? This presentation provides information and tips on how non-educators can create a standards-based education program, who might be willing to assist in the process, how to work with school districts to get into the classrooms, and how to expand the program beyond classroom visits.

299 **Title: Advancing Capacity for Behavior Change Communication and Community Engagement in Vector-Borne Disease Prevention: A Partnership Model**

Amelia Greiner Safi, alg52@cornell.edu, Emily Mader

Communicating with and engaging diverse audiences for vector-borne disease (VBD) control and prevention is an ongoing need. Many public health entities do not have the capacity or training to pursue the necessary components for effective behavior change communication interventions. We will present a model of partnering with Master of Public Health training programs that can provide communication strategy support to various entities involved with VBD control. In this model, our students work with external public health partners to develop a communication product that is tailored to the needs and barriers of specific audiences. Graduate students gain the ability to apply what they are learning about behavior change theory, audience analysis, trusted messengers, literacy, visual communication and information design to a real-world problem. Our partner entities get the support of graduate students we have trained in effective communication approaches. The process can infuse additional creativity and strategy into community engagement efforts. We will present the approach and sample products students have created to address VBDs, with an emphasis on mosquito-borne disease and management.

300 **Beyond the Booth: Increasing Engagement Through Interactive Activities**

Hester Petropoulos, hpetropoulos@ocvector.org

Booths at an outreach event are a standard tool to utilize to reach the masses, but what else can agencies add to their outreach toolbox to leave their mark when a visitor approaches? Brochures and inexpensive giveaways are the standard, but does it leave a lasting impression once the residents head home? Orange County Mosquito and Vector Control District (OCMVCD) explored the idea of advancing beyond the booth by enhancing interactive opportunities and increasing engagement time with residents through youth-focused educational games and a children's book called "Grandmother Mosquito". Learn how these activities have allowed OCMVCD to survey residents on their knowledge of vectors and services provided and gathering valuable information to further expand outreach efforts.

301 **A Historical Review of Mosquito Surveillance and Control in the United States**

Peter Obenauer, obenauerp001@yahoo.com

Mosquito control in the United States has been evolving since the first arrival of the Europeans. This presentation aims at providing examples of mosquito control over the past 200 years using historical figures and events. While technological advances in genetic engineering, computer generated dispersal equipment and site-specific insecticides have drastically changed how mosquito control is conducted, many control techniques have remained the same. This presentation will explore the good, the bad and the ugly of our war against mosquito borne diseases.

302 **Mosquito Bloodmeal Identification: Multiplexing with MinION sequencing**

Linda Kothera, lkothera@cdc.gov

Identifying animal species used as mosquito bloodmeal hosts can inform decisions on targeted locations for vector control. Traditional approaches use regular PCR followed by Sanger sequencing to generate data, which are then compared with curated sequence data in GenBank. We present an updated workflow for mosquito bloodmeal identification that uses the MinION sequencing platform by Oxford Nanopore Technologies (ONT). The MinION sequencer is a palm-sized sequencer powered by the user's laptop computer; a feature that potentially moves the generation of data closer to practitioners of vector control. The updated protocol makes use of sample multiplexing to provide faster turnaround of data comparable to the quality and accuracy of previous methods.

303 **High-throughput barcoding method of the genetic surveillance of insecticide resistance and species identification in *Anopheles gambiae* complex malaria vectors.**

Louisa Messenger, louisa.messenger@unlv.edu

Surveillance of malaria vector species and the monitoring of insecticide resistance are essential to inform malaria control strategies and support the reduction of infections and disease. Genetic barcoding of mosquitoes is a useful tool to assist the high-throughput surveillance of insecticide resistance, discriminate between sibling species and to detect the presence of *Plasmodium* infections. In this study, we combined multiplex PCR, custom designed dual indexing, and Illumina next generation sequencing for high throughput single nucleotide polymorphism (SNP)-profiling of four species from the *Anopheles* (An.) *gambiae* complex (An. *gambiae* sensu stricto, An. *coluzzii*, An. *arabiensis* and An. *melas*). By amplifying and sequencing only 14 genetic fragments (500 bp each), we were able to simultaneously detect *Plasmodium* infection; insecticide resistance-conferring SNPs in *ace1*, *gste2*, *vgsc* and *rdl* genes; the partial sequences of nuclear ribosomal internal transcribed spacers (ITS1 and ITS2) and intergenic spacers (IGS), Short INterspersed Elements (SINE), as well as mitochondrial genes (*cox1* and *nd4*) for species identification and genetic diversity. Using this amplicon sequencing approach with the four selected An. *gambiae* complex species, we identified a total of 15 non-synonymous mutations in the insecticide target genes, including previously described mutations associated with resistance and two new mutations (F1525L in *vgsc* and D148E in *gste2*). Overall, we present a reliable and cost-effective high-throughput panel for surveillance of An. *gambiae* complex mosquitoes in malaria endemic regions.

Investigating molecular mechanisms of insecticide resistance in the Eastern Democratic Republic of the Congo.

Louisa Messenger, louisa.messenger@unlv.edu

Malaria vector control in the Democratic Republic of the Congo is plagued by several major challenges, including inadequate infrastructure, lack of access to health care systems and preventative measures, and more recently the widespread emergence of insecticide resistance among *Anopheles* mosquitoes. Across 26 provinces, insecticide resistance has been reported from multiple sentinel sites. However, to date, investigation of molecular resistance mechanisms among *Anopheles* vector populations in DRC has been more limited. Adult *Anopheles gambiae* sensu lato (s.l.) and *Anopheles funestus* s.l. were collected from two sites in Sud-Kivu province and one site in Haut-Uélé province and PCR-screened for the presence of 11 resistance mutations, to provide additional information on frequency of resistance mechanisms in the eastern DRC, and to critically evaluate the utility of these markers for prospective country-wide resistance monitoring. L1014F-kdr and L1014S-kdr were present in 75.9% and 56.7% of *An. gambiae* s.l. screened, respectively, with some individuals harbouring both resistant alleles. Across the three study sites, L43F-CYP4J5 allele frequency ranged from 0.42-0.52, with evidence for ongoing selection. G119S-ace1 was also identified in all sites but at lower levels. A triple mutant haplotype (comprising the point mutation CYP6P4-I236M, the insertion of a partial Zanzibar-like transposable element and duplication of CYP6AA1) was present at high frequencies. In *An. funestus* s.l. cis-regulatory polymorphisms in CYP6P9a and CYP6P9b were detected, with allele frequencies ranging from 0.82-0.98 and 0.65-0.83, respectively. This study screened the most up-to-date panel of DNA-based resistance markers in *An. gambiae* s.l. and *An. funestus* s.l. from the eastern DRC, where resistance data is lacking. Several new candidate markers (CYP4J5, G119S-ace1, the triple mutant, CYP6P9a and CYP6P9b) were identified, which are diagnostic of resistance to major insecticide classes, and warrant future, larger-scale monitoring in the DRC to inform vector control decisions by the National Malaria Control Programme.

Latin American Symposium II

Aplicaciones de los Datos Satelitales y Avances en la Salud Ambiental: Enfoque en Una Salud

Helena Chapman, helena.chapman@nasa.gov, Laura Judd, John Haynes

El surgimiento de amenazas para la salud global, como la propagación de enfermedades transmitidas por vectores, sigue como un gran desafío a los científicos y profesionales de la salud ambiental en sus prácticas cotidianas. Los factores incluyentes como los cambios climáticos, el uso de insecticidas y las modificaciones en el uso de las prácticas de la tierra dificultan el control de vectores como también el manejo clínico. Para abordar esta carga, la formación de contribuciones científicas entre diversas disciplinas científicas – el concepto de Una Salud – permite un aprendizaje compartido donde se puede identificar brechas de conocimiento para guiar futuras investigaciones y evaluar la mejor estrategia basada en evidencia para desarrollar las intervenciones para el control de vectores apropiadas y económicas que alcanzan las necesidades de líderes y actores comunitarios. Una herramienta innovadora que puede complementar las prácticas actuales en entomología y

epidemiología para el control de vectores incluye los datos satélites, los cuales pueden ofrecer información en tiempo real sobre las condiciones ambientales y la identificación de los hábitats de mosquitos. En esta presentación, se definirá el concepto de Una Salud y se describirá la importancia de formar las colaboraciones transdisciplinarias para avanzar las aplicaciones de la salud ambiental como también expandir la red profesional con los actores comunitarios. Se presentarán ejemplos donde la integración de los datos satelitales ha ayudado a conocer los cambios dinámicos de los ecosistemas acuáticos, atmosféricos y terrestres y su impacto en la salud. Finalmente, se promoverá la comunidad de práctica de GEO Salud como ejemplo de una red global con el fin de integrar las disciplinas científicas y avanzar los descubrimientos científicos en diversas aplicaciones de la salud ambiental.

306 Isolation and characterization of bacteria from populations of *Aedes aegypti* (Diptera: Culicidae) and their role associated with susceptibility to the insecticides permethrin and deltamethrin.

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Aedes aegypti mosquito (Diptera: Culicidae) is a mosquito that transmits viral diseases such as dengue, chikungunya, Zika, and yellow fever. Insecticides are one of the most used controls to limit the development and distribution of the mosquito. The indiscriminate use of these chemical agents has triggered resistance. The manipulation of the mosquito microbiota is a promising field for the development of new vector control strategies. Thus, the aim of the study was to characterize key bacteria for the survival of mosquitoes against exposure to pyrethroid insecticides (permethrin and deltamethrin) in populations of *Ae. aegypti*. Populations of *Ae. aegypti* from the locality Umán, Yucatán, Mexico was used. Suppression of the microbiota with antibiotics (penicillin/streptomycin and gentamicin) was performed. For the isolation of bacteria was performed using soy trypticase medium ($35 \pm 2^\circ\text{C}$) and its identification by MALDI-TOF. Bacteria were selected for their growth in M9 medium added with deltamethrin and permethrin to assess the response to insecticides. The selected bacteria were supplemented to larval populations of *Ae. aegypti* New Orleans with microbiota suppression and then exposed to insecticides using CL90. *Enterobacter*, *Klebsiella* and *Staphylococcus* genera were found in the populations. It was determined that the isolated species *Enterobacter cloacae* and *Staphylococcus epidermidis* could grow in media containing insecticides. When supplementing the species *Klebsiella oxytoca* to populations with modified microbiota, there were no significant differences ($P < 0.05$) in the survival of the population against the evaluated insecticides. These results are essential for a better understanding of mosquito physiology and the role of bacteria in response to insecticides.

307 Species of mosquitoes (Diptera: Culicidae) with new records in Nuevo Leon, Mexico

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We carried out a field work during three consecutive nights of each month in an annual cycle. Two Shannon traps with protected human bait were used from 17 to 22 hours per night. In total, 3,637 mosquitoes of 26 species were captured. Of which *Aedes brelandi*, *Culex corniger* and *Sabethes chloropterus* are new records. These findings are of medical and biogeographic importance, since *Sabethes chloropterus* is the vector of yellow fever in the Neotropics.

Susceptibility and resistance mechanisms in *Aedes aegypti* populations pre-intervention with pirimiphos-methyl by targeted indoor residual spraying (TIRS)

Alicia Méndez-Manzanero, mendez_m_alicia@hotmail.com, Gabriela González-Olvera, Anuar Medina-Barreiro, Azael Che-Mendoza, Oscar David-Kirstein, Pablo Manrique-Saide, Gonzalo M. Vazquez-Prokopec

We report results of the base-line insecticide susceptibility of *Aedes aegypti* populations prior to an ongoing clinical trial quantifying the epidemiological impact of targeted indoor residual spraying (TIRS) in the city of Mérida, Yucatán, Mexico. *Ae. aegypti* eggs were collected using ovitraps from clusters of the TIRS project and randomly selected. The susceptibility of *Ae. aegypti* (F1) females to permethrin, deltamethrin, malathion and pirimiphos-methyl was quantified using dose and diagnostic times with the CDC bottle bioassay. Mortality at the diagnostic time and recovered individuals at 24 hours were recorded. Mosquito populations characterized as possibly developing resistance or resistant were exposed to enzyme inhibitors (PBO, DEF, EA) to determine which enzymatic mechanisms might be involved. Resistance was observed in most populations of *Ae. aegypti* exposed to permethrin (mortalities between 29% and 91%). For deltamethrin, one population showed 62% mortality, while in the rest ranged between 93% and 100%. For permethrin and deltamethrin, between 10% and 81% mosquitoes recovered 24 hours post-exposure. For pirimiphos-methyl, the populations showed no evidence of resistance (mortality above 93% at the diagnostic time and 100% at 24 hours post-exposure). Populations showed complete susceptibility to malathion. When using enzyme inhibitors, the contribution of resistance mediated by oxidases, esterases and *gst* was detected, which suggests that the populations identified as phenotypically resistant could be sharing enzymatic mechanisms.

The Newest State Level Vector Management Association in the Americas: The Puerto Rico Vector Management Association

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Dengue and other vector borne diseases continue to be important public health threats throughout Puerto Rico. In spite of this ever-present threat, Puerto Rico's municipal vector management personnel are poorly equipped and trained. In response to this problem, in the year 2020, the Puerto Rico Vector Control Unit (PRVCU) founded the Puerto Rico Vector Management Association (PRVMA) to improve municipal workers' expertise and professional competence. The mission of the association is to promote Integrated Vector Management Program throughout Puerto Rico. To achieve this mission, the objectives are to: (1) develop capable personnel in vector control; (2) promote new research and collaborations; (3) provide strategies to the municipalities to educate communities on vector control, among others. To date, the PRVMA has more than 175 members from different sectors (municipal, private, academic, among others) representing 47 local municipalities and six additional countries (United States, Perú, Ecuador, Chile, Spain, Colombia, and Mexico). In 2022, the PRVMA hosted its second Vector Control Annual Convention and had more than 400 attendees during the three-day event. Attendees received information about *Ae. aegypti*, vector control and surveillance approaches, and demonstrations on new technologies, industry exhibit booths, and a spray equipment rodeo for equipment calibration. This year, the PRVMA counted with the participation as speakers and collaborators people from México, Colombia, and Dominican Republic. The PRVMA is modeled after other state-wide vector management associations in the US and aspires to help implement a modern

vector management program throughout Puerto Rico and the Caribbean Region. In two years, the PRVMA will be highlighted as it proudly hosts the 2025 AMCA Annual Meeting in beautiful San Juan!

310 **Survey of *Aedes aegypti* insecticide resistance mechanisms across the United States Virgin Islands in 2020**

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A study in 2015 found insecticide resistance to permethrin and malathion on St. Croix, USVI. A broader survey to detect insecticide resistance in *Aedes aegypti* was conducted in 2020 in the U.S. Virgin Islands. Mosquitoes were collected from St. Croix (n=3 sites), St. Thomas (n=3), and St. John (n=2). Eight chemicals were tested from each site: permethrin, malathion, alpha-cypermethrin, lambda-cyhalothrin, deltamethrin, etofenprox, d-phenothrin, and naled. Mosquitoes across all islands were either susceptible to organophosphates or had low levels (87-97% mortality) of resistance with a weak mechanism behind the resistance per the CDC bottle bioassay. Resistance to pyrethroids (0-97% mortality) and strength of pyrethroid resistance (0-100% mortality after 2 hours of exposure) was variable across the islands. In general, most susceptible populations were on St. John. Further testing was done to determine which resistance mechanism(s) contributed to the observed resistance. Results from enzyme and molecular assays screening for metabolic and altered target site mechanisms will be presented. Overexpression of enzyme classes and frequency of altered target site genes was detected. Field trials with formulated products is indicated to determine what application rates might be effective in each location.

311 **Modifying the Standard Bottle Bioassay: Creative Solutions for Cielo**

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Modifying the Standard Bottle Bioassay: Creative Solutions for Cielo® The bottle bioassay is a reliable, simple and rapid surveillance tool for monitoring insecticide resistance to pyrethroid and organophosphate chemistries in local mosquito populations. However, as control products with new modes of actions (MOAs), new active ingredients, and new formulation science are developed for mosquito control, the bottle bioassay methodology and technique may need to be modified to remain the universal standard for mosquito control resistance screening. For example, the standard bottle bioassay protocol of diluting an active ingredient in acetone, coating the test bottles, and then introducing mosquitoes after the bottles are dry does not lend itself to formulations featuring solid chemistries. One such active ingredient is imidacloprid, a neonicotinoid chemistry found in Cielo®, a space spray prequalified by WHO (PQT/VC Ref No.: 020-006) for use outside of the United States. As a solid chemistry, imidacloprid requires help from formulation design, including solvents and surfactants, to effectively penetrate a mosquito cuticle and deliver a lethal dose. This presentation will highlight modifications developed to preserve the utility of the bottle bioassay as a resistance screening tool for Cielo and other next-generation mosquito control formulations. This presentation will also demonstrate the importance of bottle bioassay protocol refinements in support of responsible stewardship for emerging mode of action chemistries.

312 **The effects of age on blood-feeding, blood-meal size, fertility, and sperm storage in females of the dengue vector mosquito *Aedes aegypti*.**

Catalina Alfonso-Parra, catalfonso@gmail.com, Carolina Camargo

Early reports demonstrated that most female *Aedes aegypti* are not receptive to mating until 48-72 hours after eclosion, although a small proportion of females will mate prior to this time. In the insect model *Drosophila melanogaster*, females require ~48 hours to become receptive and are poorly fertile if they mate before this time, with the remodeling of reproductive tract tissues of recently emerged females suggested to be a requirement for the optimal fertility of *Drosophila* females. Further, age-related declines in fertility have been shown in females of several insect species. Here, we examined fertility in recently emerged females (young), and in females aged 2-3 weeks (old) to assess if recently emerged females were less fertile compared to more mature females (4-day old controls). We first determined the age (in hours) when females of our laboratory strain mated. As mosquitoes require a blood-meal to develop their eggs, we also determined when females first consumed a blood-meal. We found that females took a blood-meal several hours before they began to mate, and the quantity of blood consumed increased in the first hours post-eclosion. However, recently emerged females were similarly fertile to control, 4-day-old females, although they stored fewer sperm in their spermathecae. As expected, we observed age-related declines in female fertility, with old females laying fewer eggs and storing fewer sperm compared to control females, although old females mated at similar frequencies and had similar blood-meal sizes compared to controls. Our results show that *Ae. aegypti* females do not become receptive to mating until ~48 hours after they emerge from the pupal stage, and are fertile after they become receptive to mating.

New Product Trials II/Other

313 **Complete protection time of selected topical, spatial and electronic repellent devices tested against mosquitoes in Mali and Florida**

Gunter Muller, guntercmuller@hotmail.com, Rui-de Xue

In ongoing trials in Mali, West Africa, we are evaluating total protection time of topical, spatial and electronic repellent devices in cages, release chambers and in the field. Preliminary results are showing that depending on the test environment, total protection times vary significantly. Additionally, selected topical repellents are tested in Mali and Florida on textiles including clothing and tents. The trials are ongoing and results will be presented in detail on the poster.

314 **Biological Monitoring for Green Infrastructure Planning**

Jennifer Breaux, jabreaux@nola.gov, James Beck, Claudia Riegel

Green infrastructure (GI) is an ecologically sound approach for management of urban flood water that provides multiple community benefits. A network of green and blue infrastructure projects is currently under development in the City of New Orleans. These projects will reduce subsidence and lessen the burden on city drainage systems while beautifying neighborhoods and creating social-recreational green spaces. However, improper design or lack of maintenance can cause

unintended health threats by creating conducive conditions for mosquito vectors. Water retention can increase breeding site availability, while landscape changes can affect mosquito ecology through alterations in microclimate and habitat suitability. The City of New Orleans Mosquito, Termite, and Rodent Control Board (NOMTRCB) monitors mosquitoes at four large-scale and nine lot-scale GI project sites in different stages of construction. These data will be used to track changes in vector populations over multiple stages of site development. Population changes with negative implications for public health can then be evaluated for development of site-specific mitigation strategies. Open dialogue between vector ecologists, project managers, and stakeholders is critical to ensure proper functioning of GI sites for the protection of public health.

315 **Developments in mosquito control: Past, present and future - a manufacturer's perspective.**

Raj Saran, raj.saran@syngenta.com, Mark Hoppe, Laura Vavassori

Mosquito control approaches have evolved overtime dating back to pre-historic days when humans started to live in communities and groups. At the same time mosquitoes and vector borne diseases have also evolved and spread in different parts of the world. Climate change is definitely going to intensify the spread of vector borne diseases, especially in northern hemisphere. Experience has shown that outbreaks of diseases vectored by mosquitoes such as dengue, yellow fever, malaria, west nile virus, zika, chikungunya etc. can severely affect socio-economic status of affected regions. Therefore, quick and effective control of mosquito vectors is very important. There have been several shifts and improvements in mosquito control strategies and collaborations among public, private and non-profit organizations which played a vital role in managing vector borne diseases. Here we are presenting some of the mosquito management practices as they evolved over time and how manufacturer's pivoted their product research & development activities to find new and better solutions.

316 **New Mosquito on the Block: Tracking the Emergence of Aedes Albopictus in Chicago, 2016-2021**

Alexandra Gagner, alexandra.gagner@cityofchicago.org, Claudia Blanco, Stephanie Black, Janna Kerins, Peter Ruestow

Background: Mosquito surveillance in Chicago for Aedes albopictus began in 2016 with the emergence of new disease threats in the U.S. In 2017, CDC estimated that it was unlikely for Ae. albopictus mosquitoes to live and reproduce in Chicago. We describe how Ae. albopictus have expanded across Chicago since 2016.

Methods: The Chicago Department of Public Health (CDPH) established its vector control program in 2002. From approximately June-September, adult mosquitoes are collected from more than 80 Gravid and BG-Sentinel traps placed throughout the city, identified and counted in a CDPH laboratory weekly. Counts of Ae. albopictus collected from both types of traps were mapped by trap location and year using ArcGIS to visualize the changes in spatial distribution over time.

Results: Prior to 2016, Ae. albopictus were rarely identified in Chicago. In 2016, 4,260 Ae. albopictus mosquitoes were collected from two traps at a private tire dump site in northeast Chicago. After the tires were removed in 2017, less than 200 Ae. albopictus were collected from these same traps but they

were found at three additional trap locations. In 2018, 2,012 *Ae. albopictus* were collected from 10 different traps including seven in west Chicago. Although the total amount of *Ae. albopictus* found in 2019 decreased to 837, the number of traps with *Ae. albopictus* doubled to 20 and were more dispersed throughout the city. By 2020 *Ae. Albopictus* could be found in almost every section of city and in 2021 they were present in 51 traps, representing nearly two-thirds of Chicago traps.

Conclusions: Local enhanced mosquito surveillance demonstrated that *Ae. albopictus* are now consistently found further north in the U.S. Dedicated resources to enhance local surveillance and mitigation efforts are urgently needed to combat the expansion and endemicity of vectors capable of transmitting these emerging public health threats.

317 **The Third Time is Not a Charm: The Third Year with *Aedes aegypti* in Moab, Utah**

Michele Rehbein, michele@slcmad.org, Ary Faraji, Andrea Gloria-Soria, Shanon Amsberry

Moab is a small, rural town in southeastern Utah known for the surrounding national parks and outdoor recreational opportunities. Moab receives heavy tourist traffic with nearly three million visitors annually, yet there are only 5,500 permanent residents. *Aedes aegypti* was collected in Moab for the first time in 2019, but an aggressive vector control campaign eliminated the species prior to the end of the season. However, while *Ae. aegypti* was not collected during 2020, it was collected again in 2021 and 2022. There had been no known populations of *Ae. aegypti* in Moab prior to 2019. Moab is also outside of the original predicted range of *Ae. aegypti* estimated by the CDC. The Moab Mosquito Abatement District has a small team of employees, making it a challenge to handle routine mosquito concerns on top of an invasive mosquito.

Population genetic analysis was conducted on *Ae. aegypti* specimens collected from the 2019 and 2021 infestations. Specimens from 2019 were found to likely originate from Tucson, AZ. The specimens from 2021 were found to be unrelated to the 2019 specimens, but instead came from a source related to what invaded York, Nebraska in 2019, possibly from a southcentral or southeastern U.S. population. Specimens collected from 2022 are currently being analyzed.

Additionally, funding was received in 2022 from the Western Integrated Pest Management Center to implement an educational outreach campaign and a citizen science project geared towards reducing *Ae. aegypti* in Moab. Local students and residents participated in the monitoring of container breeding mosquitoes through the use of ovitraps. Moab *Ae. aegypti* eggs collected from ovitrap surveillance sites in 2022 will be investigated for insecticide resistance.

The operations and actions that were conducted in response to *Ae. aegypti* as well as the progress of an educational outreach campaign within Moab will be discussed.

318 **Towards the mosquito tricorder: rapid, accurate assessment of mosquitoes to radically improve efficiency of vector control programs.**

Michael Reiskind, mhreiski@ncsu.edu, Brian Byrd, Scott Huffman, Stephanie Richards

Collecting information from field caught mosquitoes, including species identification, age, and infection status is laborious, fraught with the potential for errors, and often beyond the capacity of many mosquito control districts. With the support of the AMCA Research Fund, we report

here on progress made using mid-infrared spectroscopy to accurately identify to species, age, and determine infection status of mosquitoes in the laboratory. We have completed experiments with ageing across four species of vector mosquitoes and examining infection in three pathogen-mosquito systems (two viruses and a filarial worm). Our results suggest the promise of, one day, having a “mosquito tricorder” that could scan trap contents and determine species, age, and infectious status of mosquitoes in seconds.

319 **Helicopters and Hymenoptera: Methods for monitoring non-target effects of aerial
adulicides on local pollinators in Manatee County, Florida**

Jacob Hart, jacob.hart@manateemosquito.com, Samantha Ramirez, Megan Walder,
Jeffrey Davis, Mark Latham, Christopher Lesser

Pollinators in the superfamily Apoidea are a nontarget group of primary concern and their numbers continue to broadly decline in North America. Aerial adulicides used per EPA regulations are designed to minimize nontarget impacts on pollinators with special attention given to honeybees (*Apis mellifera*). The importance of native pollinators (i.e., other bees of the family Apidae, or families Colletidae, Halictidae, and Megachilidae) has led to growing scientific and public attention, cementing the need for further research. The project described here trials methods for mosquito control agencies to efficiently and effectively monitor how pollinator populations respond to the aerial application of adulticide products. To accomplish this goal, Blue Vane Traps (BVTs) were set weekly at 14 sites served by the Manatee County Mosquito Control District (MCMCD) to selectively sample hymenopteran pollinators beginning in June of 2022. Collected bees were counted and identified to the lowest possible taxa, a minimum of genus. Samples were compared against the timing and spray blocks of helicopter adulticide missions in a before-after control-impact (BACI) design. These data were additionally compared against pre and post mosquito samples to ensure the effectiveness of missions. Data analyzed to-date include 15 species of bee across nine genera and impact reports for both malathion and deltamethrin products. Though preliminary, results detect no plausible effect of MCMCD’s aerial adulticide program on local pollinator populations. This research may be of operational use to other agencies interested in monitoring nontarget effects of aerial missions and will serve to further establish trust with an environmentally conscious public.

Public Relations

320 **Customer Service for Success; don’t ignore the phone**

Danta Smith, dmsmith@trmvc.com, Edward Horvath

A mosquito control operation doesn’t always need full-time office staff to maintain good communication with clients and the public. There are multiple tactics and resources that can help your agency keep its good reputation while avoiding overwhelming your staff to the brink of breaking. Simple measures can be used to assist the calls coming in without burdening a small agency with hiring individuals that are not necessary by stretching an already thin budget.

By devoting a few hours during a work week to these simple communication methods, we kept our residents happy, in addition to helping residents from neighboring communities. By using as few as

three individuals all working full-time in the field, we survived a summer explosion of mosquito populations, kept our clients happy, more than doubled our client base, and formed good relations with residents in adjacent communities, who were not being 'heard' by their District.

Customer service is becoming undervalued in this sector, and it is to the detriment of small businesses and Districts. Many public agencies think they are not in the business to take calls or respond to complaints verbally. Keeping the public and your clients happy is imperative for a small business or public agency to survive and can be achieved simply by utilizing these good communication and technological skills.

321 **All Aboard the Campaign Train: Getting Cities En Route to Effective Mosquito Control Outreach**

Heather Hyland, hhyland@ocvector.org

Take a journey on the campaign train with the Orange County Mosquito and Vector Control District (OCMVCD) to explore the latest campaigns implemented by OCMVCD to increase participation by cities in the County. Learn how OCMVCD's train avoided derailments and delays through collaboration, planning and evaluations. This presentation will highlight the challenges and advantages of getting stakeholder all aboard the mosquito express and help OCMVCD in building trust with the community and reaching a new level of outreach and engagement.

322 **The broad-spectrum approach to public outreach at the Lee County Mosquito Control District**

Eric Jackson, jackson@lcmcd.org, Jamie Fowler, Amy Lucas, Ian Sharp

The Lee County Mosquito Control District (LCMCD) in Southwest Florida uses an array of methods to maximize public outreach to residents and visitors of our subtropical paradise, yet prolific mosquito factory that is Lee County, Florida. School-based education programs, digital media campaigns, social media, news media interviews, District tours, weekend outreach events, and traditional billboard campaigns are tools regularly used by the District to inform, educate, and empower the public. With Lee County's booming population growth and year-round tourist industry, more and more people are residing or visiting the area and may have little understanding of the scope and operational tempo of mosquito control in Southwest Florida. A multi-faceted approach to outreach is essential to ensure that as many people as possible learn the importance of protecting public health in our community and hear LCMCD's story. This presentation will cover the various aspects of the District's approach to public relations and efforts made to educate the public about mosquito control.

323 **Wing Beats: A New Era**

Dennis Moore, dmtrinity@outlook.com

Wing Beats is an international publication for mosquito and vector control produced by the Florida Mosquito Control Association (FMCA) and is an official publication of the American Mosquito Control Association (AMCA). The publication is supported by advertisers and is mailed to nearly 4,000 individuals. Wing Beats is also available online on the AMCA and FMCA websites for members to view.

Wing Beats is published quarterly and is written and produced by those in various professions associated with mosquito control, research, and administration. Our goals are to keep interested parties informed on matters related to mosquito and vector control and disseminate information to educate and raise the level of the mosquito control profession.

Wing Beats seeks interesting technical and field-related articles about mosquitoes, mosquito control and other vector related topics. The articles need not be “scientific” in nature and vary in size from just a page or two, to multiple pages. Longer articles tend to be the most interesting and are encouraged if the additional space is warranted. We encourage the submission of manuscripts and provide guidance for authors. Author guidelines are available to help both authors and our editorial teams.

There are several significant changes to Wing Beats to report to our readers which will be outlined in the presentation.

324 **Mosquito Watch: Educating and Mobilizing Neighborhoods to Take Action Against Mosquitoes**

Caroline Gongora, cgongora@GLAmosquito.org

Mosquito abatement districts and public health agencies across the United States are facing an array of challenges to concurrently control native and invasive mosquitoes, especially the invasive *Aedes* mosquitoes. The *Aedes* mosquitoes not only pose a threat to public health, but they are changing the way of life for millions of Los Angeles residents with their aggressive day-time biting behavior. With nearly six million residents and an established invasive *Aedes* population spreading to new areas each year, the Greater Los Angeles County Vector Control District (GLACVCD/District) has been experiencing a high influx of individual/ neighborhood property inspections requests from its constituents. In response to the increased demand for property inspections and door-2-door outreach associated with *Aedes* mosquitoes, the District developed and launched Mosquito Watch, a neighborhood mosquito control program. Mosquito Watch provides the opportunity for residents, Homeowner associations, and property managers to partner with GLACVCD to educate and mobilize their neighbors or tenants to take action against mosquitoes. This presentation will discuss how Mosquito Watch has helped alleviate the need for District staff to conduct property inspections and door-2-door outreach by partnering with constituents to raise awareness and motivate behavior change.

325 **The Proof is in the Pole Banners**

Meagan Luevano, meaganl@placermosquito.org

The history of outdoor advertising dates back to the 1850s and street railways. It’s evolved from bill posters to street furniture, transit wraps, pole banners, digital billboards, and place-making experiences. But why does it work? Regardless of how someone consumes their media, outdoor advertising grasps attention and immerses consumers in visual and memorable experiences. 70 percent of a person’s day is spent outside of the home and in Placer County 140 thousand residents are commuters. This year, we geotargeted neighborhoods where West Nile virus risk was high the previous season, to encourage repellent use while shopping at supermarkets with parking lot pole banners. Our summer campaign ran for three months, June through August with two different messages aimed at increasing impressions and motivating residents to take action. Outdoor advertising was a great use of our limited advertising budget to reach a wide variety of Placer County residents on the road.

Mosquito Moment: Creating our own Vector News Source to Notify Cities and Residents

Helen Kuan, hkuan@glamosquito.org

With the establishment of the internet and social media platforms like Twitter, Instagram, and Facebook, now more than ever, people have access to an array of information and are exposed to a wide spectrum of continuous marketing. Mosquito abatement districts such as the Greater Los Angeles County Vector Control District (GLACVCD/District) are facing challenges to reach and share their mosquito control messaging to their constituents on social media platforms because they are competing with other public health messaging, especially during the COVID-19 pandemic. In order to share important information that directly impacts cities and residents, the District launched a media news source, Mosquito Moment, with the goal to disseminate public health information about vectors and vector-borne diseases and increase communication to communities. This presentation will discuss the creation of Mosquito Moment as a medium and educational tool to help increase agency visibility, alert stakeholders of pressing vector news, and engage residents to take part in the shared responsibility of vector control.

Poster Session

P-1 Sterile Insect Technique (SIT) for Individual Property Owners: A Review of the MosquitoMate Experience

Karen Dobson, kdobson@mosquitomate.com

Traditionally, methods such as the Sterile Insect Technique (SIT) are applied by government agencies across large areas. A recent report however, demonstrated that the localized, single point release of incompatible males onto an individual property could reduce the *Aedes albopictus* (Asian Tiger Mosquito) mosquito population. Subsequently, the EPA approved *Wolbachia pipiens* as a pesticide (i.e., 'ZAP Males') to induce cytoplasmic incompatibility and reduce *Ae. albopictus* egg hatch. A four-year pilot study was performed at multiple urban and suburban residences and businesses in Kentucky, to examine the application of incompatible ZAP males at individual, non-contiguous, privately-owned properties. The goal of the study was to examine unknowns including: 1) individual willingness to pay for ZAP male mosquitoes to be repeatedly released into their properties throughout the summer, 2) whether property owners found the presence of the male mosquitoes to be an annoyance, and 3) the perceived efficacy relative to traditional chemical approaches.

P-2 Biodegradable Vector Control System

Thomas Kollars, tkollars@liberty.edu, Kathryn Kollars, Lee McPhatter, Mark Carder

The Biodegradable, Visual, Olfactory, and Chemical (BVOC) device for vector control was tested for its ability to attract and control mosquitoes, meet DoD thermal and camouflage criteria, and applicability for deployment by air and hand. The BVOC device is composed of non-toxic biodegradable composite materials that can be formed into various containers and sizes. The BVOC, with pesticides can be deployed from the air, by air gun, and by hand. The BVOC can be camouflaged to match the

military operational environment and incorporates low thermal detectability, and incorporates biodegradable, visual, olfactory, and chemical components to attract and control mosquito populations and potentially other vectors. The BVOC offer s several advantages over current technologies; 1) reduces the impact on the environment, 2) targets adult and larvae mosquitoes with less pesticide ,as it is retained in a bait station and reduces impact on non-target organisms, 3) can be used for collection of mosquito species, 4) reduces risk to military personnel by blending into the environment (visual and IR) and eliminates the need to refill or collect the device. The device is designed for safety of military personnel and requires no specialized training. The BVOC can be used for control and surveillance of mosquitoes and other vectors from tree level to ground level and has both military and civilian applications. The BVOC research was funded by the US Army SBIR # W81XWH19C0091.

P-3 Entomological, epidemiological, and climatological investigation of the 2019 Dengue Fever outbreak in Gewane District, Afar Region, North-East Ethiopia

Louisa Messenger, louisa.messenger@unlv.edu, Wondemenek Mekuriaw, Solomon Kinde, Bezabih Kindu, Yibeyin Mulualem, Girma Hailu, Araya Gebresilassie, Chalachw Sisay, Fitsum Bekele, Hiwot Amare, Mesfin Wossen, Adugna Woyessa, 12:00:00 AM, , ,

Dengue Fever (DF) is an important arthropod-borne viral infection, which has repeatedly occurred as outbreaks in eastern and northeastern Ethiopia since 2013. A cross-sectional epidemiological outbreak investigation was carried out from September - November 2019 on febrile patients (confirmed malaria negative) who presented with suspected and confirmed DF at both public and private health facilities in Gewane District, Afar Region, northeastern Ethiopia. Entomological investigation of containers found in randomly selected houses belonging to DF positive patients was undertaken, to survey for the presence of Aedes larvae or pupae. A total of 1,185 DF cases were recorded from six health facilities during the 3-month study period. The mean age of DF cases was 27.2 years and 42.7% of the cases was female. The most affected age group was 15-49 years (78.98%). However, the attack rate (AR) was highest in the 49+ age group (134.2), though this should be interpreted cautiously, as this is likely a function of the small denominator, (only 2.9% of the total population), and is not necessarily an indication of increased risk among this group per se. A total of 162 artificial containers were inspected from 62 houses, with 49.4% found positive for Aedes larva/pupae. Aedes mosquitoes were mostly found breeding in buckets/bowls, clay jars, plastic tanks, and tires. World Health Organization entomological indices classified the study site as high risk for dengue outbreaks (House Index=45.2%, Container Index=49.4% and Breteau Index=129). Timeseries climate data, specifically rainfall, was found to be predictive of attack rate ($p = 0.035$). Study findings highlight the importance of vector control to prevent future DF outbreaks in the region. The long-term storage of uncovered, stagnant drinking water by community members and changing climactic conditions may have also contributed to the occurrence of this outbreak.

P-4 IPM Working Group: Mosquito BEACONS - Biodiversity Enhancement And Control of Non-native Species

Michael Riles, mriles@central.com, Dan Killingsworth, Valerie Nguyen, Ana Romero-Weaver, Yasmin Tavares, Amely Bauer, Bryan Giordano, Benjamin Allen, Elmer Gray, Lindsay Campbell, Yoosook Lee

In support of NIFA-funded Southern IPM Center, a new IPM working group called Mosquito BEACONS (Biodiversity Enhancement And Control of Non-native Species) was established in 2021. This working group is the first of its kind to bring together representatives from academia, industry, and government agencies to raise awareness of invasive mosquito species issues, develop targeted and consistent public health messaging, generate training opportunities, and plan integrative research proposals. Collectively, we cover Alabama, Mississippi, Louisiana, Florida, Georgia, North Carolina, South Carolina, Texas, and Puerto Rico. These states have serious needs for mosquito surveillance and control in general not just for invasive species. Our members are in a position of leadership in the mosquito control and research profession of each state and thus have a forum to influence stakeholders for IPM implementation in the corresponding region. Therefore, the Mosquito BEACONS working group has great potential to be used as a model working group in the other IPM regions nationally and internationally. Stakeholder meetings and capacity surveys were utilized to identify gaps in surveillance and knowledge. The survey results are being utilized to develop relevant training opportunities through our annual workshop or on-demand contents. Our working group model can be utilized in other regions not just to promote invasive species surveillance and awareness but areas of focus such as engaging with stakeholders involved in genetic biocontrol.

P-5 Are there good mosquitoes? A preliminary exploration of mosquito pollination

Christopher Vitek, christopher.vitek@utrgv.edu, Ashley Guerrero, Satinderpal Kaur, Rupesh Kariyat, Julie Mustard

While it has been hypothesized that mosquitoes may play a role in pollination, there have been only a few studies to assess the role of mosquito pollination in the field. We conducted a preliminary study to assess if pollination efforts can be observed in wild mosquito populations by viewing pollen grains on collected mosquitoes. Carbon dioxide baited ABC light traps were set in a university pollination garden to collect mosquitoes that may have recently been visiting the flowering plants. Traps were set over 9 nights, with varying attractants. A total of 279 mosquitoes were collected, sorted, and stored at 4°C. Using a stereomicroscope, an initial survey of collected mosquitoes was used to identify and separate mosquitoes that may have pollen grains on their body. Twenty mosquitoes were separated out due suspected pollen grains on their body. This number was later reduced to ten after a secondary examination. Another 7 mosquitoes were separated from the collection pool because of unusual artifacts identified on their body, which may potentially be mites. These seventeen mosquitoes were then identified to species using a dichotomous key. These mosquitoes were then imaged utilizing a Desktop Scanning Electron Microscope. DEM images were captured of mosquito body parts focusing on the legs, thorax, and head regions. While pollen was initially thought to have been observed on some mosquitoes, SEM images have not revealed any pollen grains yet (scanning is ongoing). We assess the viability of this method for exploring pollination capabilities in mosquitoes. Future surveys are planned to further assess and quantify potential pollination efforts by mosquitoes, ideally focusing on efforts to collect male mosquitoes or utilizing bar-coding to identify pollen grains more accurately. Using these tools, future quantification of pollination efforts may be feasible to better determine the role mosquitoes may play in plant pollination.

P-6 Puerto Rico Vector Control Unit's Large and Growing Integrated Vector Control Program

Nexilianne Borrero, nborrero@prvectorcontrol.org, Grayson Brown, Julieanne Miranda-Bermúdez, Joanelis Medina, Cristhian Sánchez-Rolón

Puerto Rico Vector Control Unit (PRVCU) promotes an island-wide program of Integrated Vector Management (IVM) combining vector control activities, vector and arbovirus surveillance, and community engagement and education – all focused on *Aedes aegypti*, the principal vector of dengue, Zika and Chikungunya in Puerto Rico (*Ae. albopictus* is not present in PR). The PRVCU has operated a 2,000-trap vector surveillance network since 2016. Since 2020, we have processed 800 – 1,500 mosquito pools/week through our in-house molecular laboratory PCR system. Active virus surveillance through mosquito pool testing often detects arbovirus circulation in areas where no human cases have been reported.

The Unit maintains a strong formal Quality Assurance program on its data collection and reporting system so that the data are of maximum reliability. These data are easily accessible to users of various classes (research collaborators, municipal and territorial health departments, press and media, etc.) through class-specific dashboards. Each year, the Unit also hosts visiting students (graduate and undergraduate), research collaborators, and numerous scientific guests and visitors. The Unit will be prominently highlighted at the 2025 AMCA Annual Meeting in San Juan, Puerto Rico. This poster presents the program and its results realized since 2016.

P-7 Population structure of the mosquito *Aedes aegypti* in Saudi Arabia

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Aedes aegypti (Linnaeus, 1762) is the main vector of dengue and many other viruses worldwide. This species is expected to originate off the coast of East Africa, in islands in the Indian Ocean and is well established today all over the world creating cases of yellow fever, dengue, chikungunya, and Zika. In Saudi Arabia, this species has been known in the southwestern regions since 1956. Here, *Aedes aegypti* populations, from highland on the board of Yemen to western-north, Madinah, were collected between 2019 and 2022. Microsatellite markers were used from previously published studies. A significant level of genetic structuring was found in Saudi Arabia populations. The African-like population was reported from the highland area in Jazan region, and obvious admixture was detected in the lowland of Jazan to Sahil. Bayesian clustering analysis points to $K = 2$ in Saudi Arabia using the Evanno method. Jazan highland population clusters with Uganda population (from Zika forest) while Western populations (Makkah, Jeddah, and Madinah), including Najran, cluster with Thailand. This clustering was supported by PCA and DAPC. Results of the Mantel test reveal no signal of isolation by distance in Saudi Arabia populations. Our findings are the first comprehensive study on the population genetics structure of *Ae. aegypti* from the Arabian Peninsula. This baseline informations of population structure in the country have implications for a potential future trial of the Wolbachia method for dengue.

P-8 Surveillance of *Aedes* mosquitoes in District Poonch, Azad Jammu and Kashmir

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Mosquito-borne diseases pose a huge pressure on health department around the globe. Among these diseases, dengue and zika are the most important because of the unavailability of their vaccine and global dispersal of the *Aedes* spp. Surveillance of these species is crucial to combat the

threats of associated deadly diseases and is the worldwide adopted strategy. To date, there is no record of these species in the district Poonch. Ovitrap surveillance is one of the most important and cost effective tool to detect the presence of *Aedes* spp. Therefore, in this study ovitraps were placed on different localities of district Poonch around the houses, internal and out-of-doors. Internal refers to those structures under a roof, while are those out of doors mean outside the roof, however within the immediate surrounding area of premises. Collected specimens were raised in the laboratory following the standard protocols, and were recognized up to species, using the available keys and literature. The Ovitrap index (OI) was calculated for each locality. Results showed that the maximum eggs were collected in the Aug-19 (13090). There were strong positive correlation between rainfall, temperature and humidity and total number of eggs. This study provides the evidences of presences of *Aedes* species in the selected areas and demands for future studies regarding the surveillance and control of *Aedes* species.

P-9 Lyme Disease Vector Geographic Range Expansion: South Carolina is a Battleground of Northern and Southern *Ixodes scapularis* clade colonization into new ecosystems

Anna Dill, acdill@email.sc.edu, Melissa Nolan, Kyndall Braumuller, Kia Zellars

Recent evidence indicates two subclades of blacklegged tick (*Ixodes scapularis*) exist in the USA: a northern and southern clade. Northern clade ticks exhibit more aggressive host seeking characteristics, making this subclade a public health priority. With factors such as climate change, urbanization, host movement, and variations in ecological populations, South Carolina has become a battleground for this Lyme disease vector, with both subclades encroaching along the Appalachian Mountains or Atlantic coast. Small genetic difference between the ticks migrating from the south versus the north may help researchers make important predictions about their settlement in the southeastern USA, and what implications this may have on public health and vector control agencies. The current study leverages 450 banked *Ixodes scapularis* tick samples from across South Carolina to employ a multi-year phylogenetic analysis to detail subclade spatial-temporal distribution. We hypothesize the state will become divided with established northern clade *I. scapularis* in the Upstate region, southern clade *I. scapularis* in the low country region, and focal variances of both clades in the PeeDee and Midlands regions dependent on underlying deer population abundance and environmental characteristics.

P-11 Community College and Local Agency Cooperation to Evaluate Mosquito Populations and Increase Undergraduate Research

Marcus Baber-Newton, mababernewton@actx.edu, Dustan Francis, Teresa Gaus-Bowling, Crystal Moss, Jacob Price

Due to climate change and the introduction of mosquito-vectored viruses into new areas of the United States, control and monitoring of mosquito populations have gained increased importance. To help aid the Texas Health Department and increase opportunities in student undergraduate research, a cooperation was created between the City of Amarillo Environmental Health and Amarillo College to evaluate mosquito populations and identify the presence of Arthropod-borne viruses. Working along with the City of Amarillo Environmental Health, a group of students collected and evaluated mosquitos from five locations within Amarillo, TX. Traps were set up at each location for 5 d and the collected mosquitoes were then evaluated for population number, species identification, and nucleic acid extraction. Extractions were evaluated for the presence of the mosquito-vectored viruses

West Nile virus, Eastern equine encephalitis virus, and St. Louis encephalitis virus using Real-Time RT-PCR following the National Arbovirus Surveillance Network protocols. A total of 65 mosquitoes were collected with multiple mosquito species identified from each site including three mosquito species previously unknown to the area. Of the mosquitoes collected, 55.4% were female which requires a blood meal for reproduction and can subsequently transfer viral pathogens. However, no viral pathogens were detected from the mosquitoes collected during the study. These data were sent to the Amarillo Department of Environmental Health and included in the National Arbovirus database. This study is the first cooperation between Amarillo College and the City of Amarillo Department of Environmental Health. It provided students with networking opportunities, and experience in insect population evaluation and molecular diagnostic techniques. These data also helped to guide vector control programs while providing undergraduate student research experience.

P-12 Effect of Mosquito Age and Specimen Storage Duration on Aedes aegypti RNA-based Assays

Nicole Foley, tsv4@cdc.gov, Anna Drexler, Linda Kothera, Saul Lozano

We evaluated the effect of storage duration of *Aedes aegypti* on the ability to extract viable RNA for molecular age grading. Colony *Ae. aegypti* (Rockefeller strain) adults reared in incubators to 4 d, 10 d, 21 d, and 27 d were killed and stored at 28°C for 1 h, 2 h, 5 h, 8 h, 24 h before RNA extraction. Reverse transcription-PCR was used to detect three genetic markers of age and one control gene. The Cq values from the PCR and the RNA Integrity Numbers were used to determine the quality of RNA from specimens at various ages and storage durations. Thus far, initial outcomes demonstrate substantial RNA degradation at 8 h and 24 h timepoints for all ages tested with an inability to detect age related genes. These results will be used to validate molecular age grading methodologies for *Ae. aegypti* and inform collection and storage of field specimens destined for molecular assays.

P-13 Taking Flight; Utilizing Unmanned Aircraft Systems in Delta Mosquito and Vector Control District

Hector Cardenas, hectorcardenas@deltamvcd.org, Mustapha Debboun

Delta Mosquito and Vector Control District (DMVCD) is an agricultural basin of southern San Joaquin Valley in California. Corn, one of the main crops in the District, is densely grown through flood irrigation which is the perfect *Culex quinquefasciatus* Say breeding ground. This abundant mosquito population results in an increase in West Nile virus (WNV) positive pool numbers in the District. From 2017 to September 2022, DMVCD had an average of 457 positive WNV mosquito pools per year, with many found to be associated with agriculture corn crops. This crop is difficult to treat and traditional ground application methods have had minimal impact in reducing mosquito population abundance. To address this problem, DMVCD adopted new aerial application techniques utilizing Unmanned Aircraft Systems (UAS) to reach these inaccessible areas by land. The DMVCD's goal in establishing a UAS Program was to reduce WNV transmission by integrating safe and efficient aerial applications into our Integrated Mosquito Management Program.

P-14 Finding the Right Mix: A Comparison of Yeast Fermentation Solutions as an Alternative Carbon Dioxide Source for BG-Sentinel Traps

Mark Nakata, mnakata@deltamvcd.org, Andrea Troupin, Crystal Grippin, Javier Valdivias, Mustapha Debboun

Adult mosquito surveillance plays an essential role in allowing mosquito control programs to target control efforts by monitoring populations and arbovirus activity. Carbon dioxide (CO₂), an olfactory cue for host seeking mosquitoes, in the form of dry ice, compressed gas, and yeast fermentation is widely used as an attractant for BG-Sentinel traps. Dry ice and compressed gas are challenging to use in mosquito surveillance programs because of their high cost to implement on a large-scale, difficulty to obtain during supply shortages, and safety concerns when used in public areas. As the demand for an alternative CO₂ source increases, commercially available yeast fermentation solutions are now being marketed. Since 2018, Delta Mosquito and Vector Control District (DMVCD) has used an in-house yeast fermentation solution as the CO₂ source for BG-Sentinel traps from locally acquired ingredients as a safe and affordable alternative. This study investigated adult mosquito capture efficacy of DMVCD's yeast fermentation solution to a commercial solution. The BG-Sentinel traps were set daily for three weeks at five suburban locations. Each trap set contained a BG-Lure, Elitech RC-51H temperature and humidity data logger, and rotated between: DMVCD yeast solution with non-insulated container; DMVCD yeast solution with insulated BG-Pro Bag; BG-CO₂ Powder with non-insulated container; BG-CO₂ Powder with insulated BG-Pro Bag; or no CO₂ source. Daily mosquito population abundance was compared between the combination of different yeast fermentation solutions and containers. As DMVCD's Surveillance Program continues to grow, collection methods must be closely examined as the District sets over 150 BG-Sentinel traps a week in 2022. By improving the yeast fermentation process, DMVCD can implement more effective control methods based on collection data from a proven CO₂ source.

P-15 **A Valiant Attempt at Surveying Mosquito District Practices of California**

Andrea Troupin, atroupin@deltamvcd.org, Crystal Grippin, Mark Nakata, Javier Valdivias, Mustapha Debboun

Mosquito control programs throughout the United States rely on various methods for mosquito surveillance, control methods, and vector-borne disease testing. Strategies and protocols vary widely among Districts due to differing regional needs, budgets, staffing, experience, and backgrounds. This information is often only shared through webinars, conferences, and word of mouth. A survey was sent out to California's mosquito control organizations asking for general information about mosquito trapping, field operations, and disease testing. Results from participating Districts were compiled and categorized. The development of standardized protocols will allow for a deeper understanding of various strategies. This results in Districts being able to compare surveillance, control methods, and vector-borne disease testing to better manage mosquito populations and vector-borne disease risk.

P-16 **Evaluation of Aedes larvae Habitat Characteristics in South Punjab Pakistan**

Shafia Saba, sabashafia@yahoo.com, Unsar Naeem-Ullah, Shafqat Saeed, Ishtiaq Rajwana, Ata-ur-Rehman Khan, Dil awez

Aedes species are recognized as vectors for a variety of viral diseases. *Aegypti* and *Albopictus* are the two species to disseminate viruses that cause Zika virus, chikungunya, dengue fever, yellow fever, West Nile fever, and encephalitis most frequently.

In current study, habitat characters of both species were premeditated by collecting *Aedes* larvae from the potential breeding sites and calibrated the physical and chemical characteristics of breeding sites.

Aedes aegypti was found to be more broader range species in terms of the parameters studied. Maximum population of both species was found from clear-foul type of water along with other types including turbid-foul, turbid and clear. Both species were generally present in colorless water and least presence was observed in muddy water. EC range for *Aedes aegypti* was 268-9802 and for *Aedes albopictus* it was 430-6876. Similarly, pH range was 7.9-12.6 and 7.3-10.6 for both species respectively. TDS range was 134-4901 and 215-2630, water temp. range was almost similar for both species. *Aegypti* was dominant in urban dwellings while *Albopictus* was overriding in rural settings. It is pertinent to mention that at one place among 266 *Aedes* larvae positive sites, both the species were found to be cohabiting. The water quality was clear-foul, EC 2186, pH 9.5, and TDS 1093. Coinhabiting of both species was observed at rural area in an indoor breeding site.

Current findings suggest that selection of mosquito breeding habitats by *Aedes* mosquitoes is significantly influenced by physical and environmental factors. These preferences might be used to create novel methods of preventing oviposition. and eventually overcoming the disease burden.

P-17 The Effects of a Sublethal Dose of an Insecticide on Mosquito Predators

Erin Cloherty Duvernay, ercloherty@nola.gov, James Ottea

Bacillus thuringiensis israeliensis (Bti) is a bacterium used as a larvicide to control mosquito populations. We hypothesized that larval *Aedes aegypti* exposed to sublethal concentrations of Bti and fed to predator, *Toxorhynchites rutilus* larvae, would result in *T. rutilus* mortality. No significant mortality was observed. Further investigations are needed to determine effects in the adult populations of *T. rutilus* that consumed mosquito larvae exposed to Bti.

P-18 The socio-cognitive field of the sterile insect technique (1979-2022):Insights into its specific labor division form and some other aspects

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We apply socio-semantic analysis tools to a corpus of abstracts and metadata of scientific articles to characterize the field of the sterile insect technique (SIT). The networks and clusters produced shed light on the specific structuration of SIT, which illustrates the perenity of disciplinary boundaries as well as their overcoming with radiobiology and engineering. A specific division of labor emerges between the subfields and in the relations between nation-states. We argue that the mixed regime of knowledge production and international anchoring relate to the operations of public relations and legitimization of the technique around the research and development programs on mosquito control.

P-19 Building an IIT/SIT program at Anastasia Mosquito Control District

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Since 2017, Anastasia Mosquito Control District, in St. Johns County Florida, has been collaborating with the United States Department of Agriculture Center for Medical and Veterinary Entomology, Mosquito Mate, and the University of Florida Department of Entomology and Nematology

for evaluating Incompatible Insect Technique and Sterile Insect Technique (IIT/SIT) approaches for urban mosquito control. Support for these projects has been through funding from the Center for Disease Control and Prevention Crisis Co-Ag and the Florida Department of Agriculture and Consumer Services. Currently, AMCD is building a 6000 sq. ft. building with the capacity to do IIT and SIT. The anticipated completion of this project is in early 2023. This presentation will highlight both laboratory and field work completed by AMCD. We will also present on the design and build of the IIT/SIT building including the operational goals of this program for urban Aedes control and future directions for other nuisance and vector control targets.

P-20 Quality Control Methods implemented in the Integrated Vector Management (IVM) at Puerto Rico Vector Control Unit

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The Puerto Rico Vector Control Unit (PRVCU) is a program of the nonprofit organization, Puerto Rico Science Technology & Research Trust (PRSTRT). The PRVCU focuses on the reduction of *Aedes aegypti* in Puerto Rico and nearby islands. As part of this program, the PRVCU promotes Integrated Vector Management (IVM) which includes large-scale vector surveillance, community engagement and the use of innovative control techniques to reduce *Aedes aegypti* in Puerto Rico. The PRVCU operates a large and complex surveillance network, and our data are accessed by a wide variety of users. To ensure that these data meet the users' needs, a quality control program was begun in 2021. This Quality Control Program seeks standardization of procedures through process evaluation, protocols creation and implementation and training of personnel in charge of vector management. The results of implementing this program have been an improvement in data collection, higher employee confidence when performing their tasks, an elevated employee efficiency, and overall better compliance with the PRVCU's surveillance SOP's. This poster presents some of those results and emphasizes its potential utility to other vector management program.

P-21 What we know about mosquito control and fireflies

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While very few long-term population studies on Lampyridae exist, anecdotal evidence suggests that firefly abundance is potentially decreasing worldwide for many important species. While multiple potential causes have been proposed, namely loss of habitat and light pollution, public health pesticides are often listed as a possible factor, despite the lack of research on the topic. To help bridge this knowledge gap, we conducted a literature review to evaluate if mosquito control practices could negatively impact fireflies. To further fill in gaps, we used existing research on similar insect groups to Lampyridae to determine where potential non-target risks exist.

P-22 Knockdown resistance mutations in California: monitoring insecticide resistance in *Culex tarsalis*

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Monitoring the status of mutations linked to insecticide resistance is essential as we move forward in combating vector-borne diseases. One such mutation, kdr, currently has an unknown

status in California populations of *Culex tarsalis*, a vector of West Nile virus and St. Louis encephalitis. By assessing the status of this mutation in *Cx. tarsalis*, we can better understand the evolutionary implications of such mutations and how pesticide usage may lead to evolutionary pressure. To investigate these mutations, we utilize Quantitative Polymerase Chain Reaction, identifying the allele at the *kdr* mutation site for individual samples, where we expect a leucine to phenylalanine or serine switch. The samples included in our analysis range from Southern California (Coachella Valley) to Northern California (the Sutter-Yuba region) and begin in 1990 and continue to 2 different time points (2007-2009 and 2016-2018) through samples collected in 2022. Continued work in our lab on developing a qPCR melt curve assay has led us to efficiently and accurately identify each individual's *kdr* genotype by designing primers specific to the *kdr* mutation site. The primary goal of this project is to evaluate the current status of the *kdr* mutation in *Cx. tarsalis* and the relation to past allele frequencies. Additionally, with access to the California Department of Pesticide Regulation's data on pyrethroid application in California, we hope to identify whether there is a correlation between pyrethroid usage by different constituencies and changes in allele frequency over time.

P-23 Establishing the Window of Selection (WoS) and the level of resistance of knock down resistant (*kdr*) mutations for *Aedes aegypti* mosquitoes topically treated with deltamethrin

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Perpetual use of insecticides has led to the emergence and persistence of insecticide resistance. Insecticide resistance is a major threat that prevents effective mosquito population control and consequently results in continued mosquito-borne disease transmission. Knockdown resistance (*kdr*) confers resistance against pyrethroids – the main insecticide class used for disease prevention. Selection for *kdr* occurs within the Window of Selection (WoS), which establishes the concentration range of xenobiotic that exerts positive selection for resistance. Fundamentally, selective pressure is highest within the WoS when all genotypes containing resistant alleles – heterozygous and homozygous-resistant organisms – survive. However, heterozygous mosquitoes are infrequently included in resistance studies and models. Additionally, male mosquitoes are less included because they are of lower epidemiological relevance. In this study, we establish the WoS of deltamethrin for *Aedes aegypti* *kdr* strains (including heterozygotes and males) using topical bioassay application and establish the level of resistance (LoR) of each strain and sex. Strains included in this study originate from Florida where deltamethrin has been historically used, and contain zero, one, or two alleles of *kdr* mutations V1016I and F1534C. Results determine the upper bound of the WoS to exceed the amount of deltamethrin recommended for mosquito control by the U.S. Environmental Protection Agency. Therefore, we can assume applications following these recommendations are selecting for *kdr*. The LoR was significantly different between the heterozygous and homozygous-resistant strains, supporting the inclusion of heterozygotes in future research and modeling. Lastly, results show no significant difference in the LoR between males and females of the same strain when comparisons are mass-relativized. This suggests that males could be used for resistance surveillance, increasing their flexibility. Continuation of this work will improve our understanding of which insecticide treatments create the greatest selection for resistance and will improve our modeling of insecticide resistance evolution.

P-24 The status of La Crosse virus in La Crosse, Wisconsin

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La Crosse virus (LACV) can cause encephalitis and has a lethal history in La Crosse, Wisconsin, with children being most at risk. The first human case of LACV was discovered in 1964 in La Crosse. Surveillance of La Crosse encephalitis and mosquito control began in 1978 by the La Crosse County Health Department and ended in 2017 because diagnosed cases of the disease have declined dramatically in La Crosse County since the late 1970's. We postulated that the decline in cases could be related to successful control of the vector species in the area or a change in the virus associated with more asymptomatic infections. In 2020 and 2021, I returned to historical case sites to survey for the primary vector species, *Aedes (Ochlerotatus) triseriatus*. I collected mosquito eggs via oviposition traps fitted with seed germination papers during the summer in 2020 and 2021. Mosquitoes were reared to adulthood, identified to species and then tested for LACV via RT-PCR. None of the mosquitoes collected in 2020 tested positive for LACV and the results for 2021 are forthcoming. Our results show that *Ae. triseriatus* continues to occupy the habitat provided by deciduous forests around the city of La Crosse, and that this mosquito is now competing for habitat with *Aedes japonicus*.

P-25 **ARGO trailer nurse tank for extended larvicide applications at remote sites**

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We will describe our solution to applying multiple batches of larvicide in areas where access to water for the mix tank is limited. Custom fabricated plumbing mounted to the trailer under an ARGO and to the sprayer provides technicians with an additional 100 gallons of water, which reduces the need for another vehicle, assistance, or time spent traveling to an alternative site to obtain water.

P-26 **Experimental transmission of Mayaro virus by *Aedes aegypti* mosquitoes**

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Vector competence of *Aedes aegypti* for Mayaro virus (MAYV) was determined at 20°C and 30°C for several time points. We observed a trend of increase in progression of MAYV infection and replication over time, followed by a decline during later periods. Mosquitoes were able to transmit MAYV after 3 and 15 days post infection at 30°C and 20°C, respectively. Our results suggest that temperature and time can influence MAYV progression of infection in *Ae. aegypti*.

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AMCA AWARDS

HONORARY MEMBERS

1937	Leland O. Howard (USDA)	1970	Robert L. Vannote (NJ)	1998	Eugene J. Gerberg (MD)
1938	C. C. Adams (NY)	1971	Richard W. Fay (USPHS)		Glen C. Collett (UT)
1944	Thomas J. Headlee (NJ)	1972	Christian T. Williamson (NY)	1999	Donald R. Johnson (GA)
	William B. Herms (CA)		Alan R. Stone (MD)	2001	Fred W. Knapp (KY)
	J. A. LePrince (USPHS)	1974	Edward S. Hathaway (LA)	2003	E. John Beidler (FL)
	Louis L. Williams, Jr. (USPHS)	1976	Theodore G. Raley (CA)	2004	David A. Dame (FL)
1948	Robert D. Glasgow (NY)	1979	John A. Mulrennan, Sr. (FL)	2005	Donald J. Sutherland (NJ)
	Willard V. King (USDA)		Thomas D. Mulhern (CA)	2006	Martin S. Chomsky (NJ)
1951	Lewis W. Hackett (CA)	1981	Austin W. Morrill, Jr. (CA)	2013	Judy Hansen (NJ)
	Robert Matheson (NY)	1983	William R. Horsfall (IL)	2013	Henry Rupp (NJ)
1955	Harold F. Gray (CA)		Anthony W. A. Brown (WHO)	2017	Sammie Dickson
1958	Louis A. Stearns (DE)	1991	Kenneth L. Knight (NC)		
1964	George H. Bradley (USPHS/USDA)	1994	Harold C. Chapman (LA)		
1965	Arthur W. Lindquist (KS)		Lewis T. Nielsen (UT)		
1967	Fred L. Stutz (FL)				

HAROLD FARNSWORTH GRAY MEMORIAL CITATION MERITORIOUS SERVICE TO MOSQUITO CONTROL AWARD

This now discontinued award was presented to an active member of AMCA for exceptional service to the Association and to mosquito control or related vector control.

1964 Fred C. Bishopp (DC)

DR. THOMAS J. HEADLEE MEMORIAL AWARD

This now discontinued award recognizes a living member of the Association for outstanding service to the field of mosquito control, while simultaneously commemorating the name of a deceased member.

1968 George H. Bradley (USDA/USPHS)

MEDAL OF HONOR

Next to honorary membership, the Medal of Honor is the highest award regularly given by AMCA. The only specific limitation for the Medal of Honor is AMCA membership, and nominees are selected on the basis of exceptional contributions to mosquito control or related fields. After 1982, the Board of Directors set a suggested maximum of one Medal of Honor

1972	Maurice W. Provost (FL)	1985	Norman G. Gratz (WHO)	2007	E. John Beidler (FL)
	William R. Horsfall (IL)	1986	James R. Caton (CA)	2008	David A. Dame (FL)
1973	Don M. Rees (UT)	1987	Jay E. Graham (UT)	2009	Dan Ariaz (NV)
	Thomas D. Mulhern (CA)	1988	Lewis T. Nielsen (UT)		Gary Breeden (VA)
1974	Anthony W. A. Brown (WHO)	1989	Andrew J. Spielman (MA)	2010	Mir S. Mulla (CA)
	Donald L. Collins (NY)	1990	Glen C. Collett (UT)	2011	Dave Brown (CA)
1975	Daniel M. Jobbins (NJ)	1991	Harold C. Chapman (LA)	2012	Sammie L. Dickson (UT)
	Arthur W. Lindquist (USDA)	1992	D. Bruce Francy (CO)	2013	Wayne Crans (NJ)
1976	Austin W. Morrill, Jr. (CA)	1993	Gilbert L. Challet (CA)	2014	Chester G. Moore (CO)
	Carroll N. Smith (USDA)	1994	Ronald A. Ward (MD)	2015	Jorge Arias
1978	James B. Kitzmiller (FL)	1995	T. Wayne Miller (FL)	2016	Graham White (FL)
	William D. Murray (CA)	1996	Marshall Laird (New)	2017	Randy Gaugler (NJ)
1979	Richard F. Peters (CA)	1997	Robert K. Washino (CA)		Allan Inman (CA)
1980	William E. Bickley (MD)	1998	John D. Edman (MA)	2018	Bill Meredith (DE)
	John N. Belkin (CA)	1999	Bruce F. Eldridge (CA)		Roger Nasci (IL)
1981	Stanley J. Carpenter (CA)	2000	Judy A. Hansen (NJ)	2020	Joe Conlon (FL)
	Roland E. Dorer (VA)	2001	Gary G. Clark (USPHS)	2021	Steve Mulligan (CA)
1982	Kenneth L. Knight (NC)	2002	Lucas G. Terracina (LA)	2022	Rui-de Xue (FL)
	William C. Reeves (CA)	2003	Robert J. Novak (IL)	2023	Ken Linthicum (FL)
1983	Harry D. Pratt (GA)	2004	James D. Long (TX)		Janet McAllister (CO)
	John A. Mulrennan, Sr. (FL)	2005	James W. Robinson (FL)		
1984	George T. Carmichael (LA)	2006	John L. Clark Jr. (IL)		

MERITORIOUS SERVICE AWARD

Given to individuals for outstanding service, the contributions of the nominees must be considered outstanding as judged by their peers. Only AMCA members in good standing who are not past presidents of AMCA are eligible. After 1982, the Board of Directors set a suggested maximum of no more than two awards per year.

1972	Charles F. Scheel (IL)	1981	A. Ralph Barr (CA)	2002	Thomas G. Floore (FL)
	Donald L. Collins (NY)		Gilbert L. Challet (CA)		Sherry McLaughlin (TX)
	Theodore G. Raley (CA)		Edgar A. Smith (VA)	2003	Wayne L. Kramer (NE)
1973	Francis P. Creadon (CA)	1982	Hugo A. Jamnback (NY)		John L. Clarke, Jr. (IL)
	Vernon Conant (NJ)		Donald R. Johnson (GA)	2004	Yadira N. Rangel (Venezuela)
	Austin W. Morrill, Jr. (CA)		Harold D. Newsome (MI)		James W. Robinson (FL)
1974	Leslie D. Beadle (USPHS)		James V. Smith (GA)	2005	Major S. Dhillon (CA)
	John H. Brawley (CA)	1983	Richard F. Darsie (CO)		William H. Meredith (DE)
	John W. Kilpatrick (GA)		Ronald A. Ward (DC)	2006	William J. Sames (WA)
	T. Oscar Fultz (GA)	1984	Samuel G. Breeland (FL)	2007	Henry R. Rupp (NJ)
	Howard R. Greenfield (CA)		Donald J. Sutherland (NJ)	2008	Allan Inman (CA)
	Paul J. Hunt (FL)	1985	John C. Kuschke (NJ)		Manuel Lluberas (FL)
	William C. McDuffie (USDA)		James R. Caton (CA)	2009	Joe Conlon (FL)
	Donald R. Johnson (GA)	1986	C. Lamar Meek (LA)	2010	Norbert Becker (Germany)
	Helen Sollers-Riedel (DC)	1987	John C. Combs (CA)	2011	Harry Savage (CO)
1975	Lewis E. Fronk (UT)	1988	Chester G. Moore (CO)		L.A. Williams (SC)
	Joseph G. McWilliams (USN)		Margaret Parsons (OH)	2012	Lal S. Mian (CA)
	Lewis J. Ogden (USPHS)	1989	John S. Billodeaux (LA)		Edsel M. Fussell (FL)
	Rajindar M. Pal (WHO)		Edgar S. Bordes, Jr. (LA)	2013	Kenneth J. Linthicum (FL)
	Kenneth D. Quarterman (USPHS)	1990	Richard D. Morton (WA)	2014	Diann Crane (MN)
	Herbert F. Schoof (USPHS)		Lucas G. Terracina (LA)		Daniel Kline (FL)
1976	Robert A. Armstrong (MA)	1991	David A. Dame (FL)	2015	Mark Latham (FL)
	Osmond P. Breland (TX)	1992	Jerry Mix (TX)	2016	Rui-de Xue (FL)
	George B. Craig, Jr. (IN)	1993	William E. Hazeltine (CA)		William Reisen (CA)
	Claude M. Gjullin (USDA)	1994	Sally A. Wagner (MI)	2017	Michael Turell (MD)
	T. Wayne Miller (FL)	1995	Frederick W. Wagner (KY)	2018	Gary Goodman (CA)
1976	Donald J. Pletsch (Mexico)	1996	Donald J. Sutherland (NJ)	2019	Angela Beehler (WA)
	Glenn M. Stokes (LA)		Ronald A. Ward (MD)	2020	Michael Riles (FL)
	Luis M. Vargas (Mexico)	1997	Roger S. Nasci (CO)	2021	Mustapha Debboun (CA)
1978	Richard C. Axtell (NC)	1997	Thomas J. Zavortink (CA)		
1979	Marco. E. C. Giglioli (BWI)	1998	James D. Long (TX)		
1980	James D. Gorman (FL)	1999	Hilton B. Munns (CA)		
1980	Donald E. Weidhaas (FL)	2000	Leroy J. Bohn (VA)		
	E. John Beidler (FL)		Dreda McCreary (VA)		
	Eugene J. Gerberg (MD)	2001	Charles T. Palmisano (LA)		

PRESIDENTIAL CITATION

The Presidential Citation recognizes individuals not eligible to receive other awards but who are eminently deserving of special recognition by AMCA. Recipients need not be AMCA members. After 1982 the Board of Directors set a suggested maximum of no more than 2 awards per year.

1980	John M. Poché (LA)	1997	Charles T. Palmisano (LA)	2012	Truc Dever (CA)
	Leslie E. Fronk (UT)		George J. Wichterman (FL)	2013	Robert Peterson (MT)
	Jesse B. Leslie (NJ)	1998	Douglas B. Carlson (FL)	2014	Salvador Rico (TX)
1981	Linda G. Raiche (CA)	1999	Charles Beesley (CA)	2015	Kristy Burkhalter (CO)
	Margaret S. Slater (NY)		Donald R. Johnson (GA)		Elizabeth Cline (CA)
1982	K. G. Nolan (NY)	2000	Peter B. Ghormley (CA)	2016	Angela Beehler (WA)
	Charles F. Scheel (IL)		David A. Brown (CA)		John Biedler
1983	Coyle E. Knowles (NY)	2001	Donald Menard (LA)	2017	Peter Connelly (FL)
1984	Ray Treichler (DC)		Joel Margalit (Israel)		Larry Smith (GA)
1985	Lawrence T. Cowper	2002	Dennis Moore (FL)	2018	Stephen Sickerman (FL)
	Janice B. Wells (NY)		Henry R. Rupp (NJ)		Isik Unlu (NJ)
1986	T. Oscar Fultz (GA)	2003	James R. McNelly (NJ)	2019	Brian Byrd
1987	Sharon A. Colvin (IL)		Robert Bonnett (MN)		Rui-de Xue
1988	Daniel D. Sprenger (TX)	2004	James R. Brown (FL)	2020	Levy Sun (CA)
1989	Fred C. Roberts (CA)	2005	Mark Newberg (IL)		Harry Savage (CO)
1990	Leonard E. Munsterman (IN)		Susan Maggy (CA)	2021	Gary Hatch (CA)
1991	James D. Long (TX)	2006	Teung Chin		Kristen Healy (LA)
1992	Charlie D. Morris (FL)	2007	Karl Malamud-Roam (CA)	2022	Catalina Alfonso-Parra (Columbia)
1993	Robert J. Novak (IL)	2008	William H. Meredeth (DE)		Tianyun Steven Su (CA)
1994	James W. Robinson (FL)	2009	Rep. Dennis Cardoza (CA)	2023	Jennifer Gordon (CO)
	Dan L. Ariaz (NV)	2010	Gordon Patterson (FL)		Ary Faraji (UT)
1995	Sally Kuzenski (LA)		Gary Clark (FL)		
1996	Carl R. Tanner (IL)		Yasmin Rubio-Palis		
	Sammie L. Dickson (UT)	2011	Angela Beehler (WA)		
			Roxanne Connelly (FL)		

JOHN N. BELKIN AWARD

The John N. Belkin Award is given for meritorious contributions to the field of mosquito systematics and/or biology and may be given to anyone judged by his peers to be worthy. Usually, a maximum of one award per year is given.

1981	Botha de Meillon (PA)	2001	John F. Reinert (FL)
1982	Lloyd E. Rozeboom (IL)	2002	Richard F. Darsie (FL)
1983	Kenneth L. Knight (NC)	2003	Richard C. Wilkerson (MD)
1984	Thomas J. Zavortink (CA)	2004	Kazuo Tanaka (Japan)
1985	Stanley J. Carpenter (CA)	2005	Ronald A. Ward (MD)
1986	Elizabeth P. Marks & John Reid (Australia)	2006	William K. Reisen (CA)
1987	James B. Kitzmiller (FL)	2008	Maria-Anice Sallum (Brazil)
1988	Allan R. Stone (MD)	2010	Daniel Strickman (MD)
1989	Pedro Galindo (Panama)	2011	Rampa Rattanarithikul, Ph.D. (Thailand)
1990	Peter F. Mattingly (UK)	2012	Maureen Coetzee, Ph. D. (South Africa)
1991	Jose P. Duret (Argentina)	2013	John F. Anderson (CT)
1992	Bruce A. Harrison (NC)	2014	Graham White (FL)
1993	Edward L. Peyton (DC)	2015	Elena B. Vinogradova (Russia)
1994	Theodore H. G. Aitken (CT)	2016	
1995	Oswaldo P. Forattini (Brazil)	2017	George F. O'Meara (FL)
1996	A. Ralph Barr (CA)	2018	Dr. L. Philip Lounibos (FL)

	Michael W. Service (UK)	2019	Norbert Becker
1997	Christine J. Dahl (Sweden)	2020	Jan Conn
1998	Ralph E. Harbach (UK)	2021	Ken Linthicum
1999	Yiau-Min Huang (DC)	2022	Chet Moore (CO)
2000	Lewis T. Nielsen (UT)	2023	John Edman (SC)
			Bruce Eldridge (CA)

MEMORIAL LECTURE HONOREE & MEMORIAL LECTURER AWARD

The Memorial Lecture Honoree must be one who has made exceptional contributions to the broad field of mosquito control during his lifetime. If there is more than one honoree in a given year, then the group must have made significant contributions as a team or equal stature in the same time frame and to the same aspect of mosquito control. The Memorial Lecturer Award is given to an outstanding speaker (one per year) to present the annual Memorial Lecture in honor of the Memorial Lecture Honoree. The Memorial Lecture Award is not limited to a member of AMCA, but the recipient should be a recognized authority in the broad field of vector control.

	HONOREE	LECTURER	TOPIC
1979	Don M. Rees	J. David Gillett	Out for blood: Flight orientation upwind & in the absence of visual clues
1980	Maurice W. Provost	Anthony W. A.	What have insecticides done for us?
1981	Leland O. Howard	Leonard J. Bruce-Chwatt	Leland Ossian Howard (1857-1950) and malaria control then and now
1982	Carlos Finlay Walter Reed William Gorgas Fred Soper	William C. Reeves	A memorial to Finlay, Reed, Gorgas and Soper as major contributors to present-day concepts essential for control of mosquito-borne viruses
1983	Harry H. Stage	Michael W. Service	Biological control of mosquitoes—Has it a future?
1984	Louis L. Williams	George B. Craig, Jr.	Man-made human disease problems: Tires & La Crosse virus
1985	Thomas J. Headlee	William R. Horsfall	Mosquito abatement in a changing world
1986	Marston Bates	A. Ralph Barr	The basis of mosquito systematics
1987	William B. Herms Harold F. Grav	Robert K. Washino	
1988	John A. Mulrennan, Sr.	Susan B. McIver	Mosquitoes, medicine & memories
1989	Brian Hocking	John D. Edman	Are biting flies gourmet or gourmand?
1990	John N. Belkin	Thomas J. Zavortink	Classical taxonomy of mosquitoes—A memorial to John N.
1991	Edward S. Hathaway	C. Lamar Meek	Les maringouins du mech: The legacy of two men
1992	Anderson B. Ritter		
1992	Sir Patrick Manson	Bruce F. Eldridge	The man we honor
1993	Willard V. King	Ronald A. Ward	Renaissance man of medical entomology
1994	Stanley B. Freeman	Mir S. Mulla	Now & in the future
1995	Maurice T. James	Wayne A. Rowley	Maurice T. James
1996	Telford H. Work	Charles A. Calisher	Telford H. Work—A tribute
1997	Stanley J. Carpenter	Lewis T. Nielsen	In honor of Stanley Carpenter
1998	George B. Craig, Jr.	Robert J. Novak	George Brownlee Craig
1999	A. Ralph Barr	Andrew J. Spielman	
2000	John B. Smith	Wayne J. Crans	
2001	William R. Horsfall	Jimmy K. Olson	
2002	Edward F. Knipling	Waldemar Klassen	Titan and Driving Force in Ecologically Selective Area-Wide Pest Management
2003	Kenneth L. Knight	Ralph E. Harbach	Mosquito systematics: From organism to molecules—A tribute to Kenneth L. Knight
2004	Donald J. Pletsch	David A. Dame	Six Decades of International Commitment
2005	William E. Hazeltine	Bruce F. Eldridge	William E. Hazeltine: Rebel with a cause
2006	William C. Reeves	Grant R. Campbell	
2007	Norman G. Gratz	Graham B. White	Remembering Norman Gratz (1925-2005) – Doyen of Vector Control
2008	Andrew Spielman	John D. Edman	
2009	Lamar Meek	Roxanne Connelly	
2010	Harold C. Chapman	Tokuo Fukuda	
2011	H.G. Dyar	Terry Klein	
2012	James D. Long	John Welch	
2013	Thomas Mulhern	Randy Gaugler	
2014	Founding Mothers of Mosquito Control	Gordon Patterson	

2015	Dr. Richard F. Darsie, Jr.	Dr. Jonathan F. Day
2016	Oscar Fultz	Joe Conlon
2017	Jimmy Olson	Bill Sames
2018	Fred Knapp	Steve Presley
2019	William Opp	Gordon Patterson
2020	Lucas Terracina	Scott Willis
2021	Lew Nielsen	Sam Dickson
		Mark Blackmore
2022	Gary Clark	Dan Kline
		Kenneth Linthicum
2023	Dan Strickman	Mustapha Debboun

INDUSTRY AWARD

Established in 1997, the Industry Award is presented to a representative of a mosquito/vector-related industry who has through his/her efforts advanced the work of mosquito and/or vector control or research.

1997	Charles T. Galley (FL)	2010	Peter Connelly (FL)
1998	William German (FL)	2011	David Sullivan (MT)
1999	Gary A. Mount (FL)	2012	Stephanie Whitman (WY)
	Daniel F. Boyd (GA)	2013	Larry Erickson (IL)
	David W. Waldron (GA)	2014	Gerry Hutney (FL)
	J. David Waldron (GA)	2015	Joe Strickhouser (NC)
2002	Robert E. Richard (TX)	2016	Terry Couch (FL)
2003	Allen W. Wooldridge	2017	Clark Wood (IL)
2004	John L. Clarke, Jr. (IL)		Malcom Williams (AR)
2005	Ernest Danko (IL)	2018	Larry Smith (FL)
2006	Willie N. Cox (IL)	2019	Peter DeChant
2007	Bob Bonnett (MN)	2020	Martin Geier
2009	Clarke Hudson (IL)	2021	Bill Reynolds
	Bill Strange (ID)	2022	Mark Newberg
		2023	John Neberz

GRASSROOTS AWARD

This award is given to recognize excellent performance and dedication by mosquito control field staff.

2005	Omar S. Akbari	Reno Washoe Country, Nevada
	Christopher Trapp	Multnomah County Vector Control, Oregon
2006	John Phelps	Mercer County, New Jersey
2008	Chris Frame	Cape May County, New Jersey
2009	Jason Craig Hardman	Salt Lake City MAD, Utah
2010	Jessica Fales	Midland County MC, Michigan
	Gary Hillsdale	Metropolitan MCD, Minnesota
	Elizabeth Vice	Butte County MVCD, California
2011	David Bruget	Kings MAD, California
	Russell Eck	Washoe County Health District, Nevada
	Phillip Henry	Butte County MVCD, California
	Levi Zahn	Williston VCD, North Dakota
2012	Mike Smith	Anastasia MCD, Florida
2013	Arturo Gutierrez	Coachella Valley MVCD, California
2013	Michael Martinez	Coachella Valley MVCD, California
2013	David Lopez	Greater Los Angeles County VCD, California
2013	Martin Serrano	Greater Los Angeles County VCD, California
2014	Dell Boyd	Butte County MVCD, California
	John McCready	Jackson County VCD, Oregon
	Gaby Perezchica-Harvey	Coachella Valley MVCD, California

2015	Geneva Ginn	Coachella Valley MVCD, California
	Kevin Hill	Pasco County MCD, California
	Richard Ortiz	Coachella Valley MVCD, California
	Terry Sanderson	Lake County MVCD, California
	Melissa Snelling	Coachella Valley, MVCD, California
2016	Patrick Morgan	Indian River MVCD, Florida
	Janet Nelson	Northwest MVCD, California
	Richard Weaver	Anastasia MVCD, Florida
2017	Hailey Bastian	Shasta MVCD, California
	Gregorio Alvarado	Coachella Valley MVCD, California
	Aaron Lumsden	Butte County MVCD, California
	Danny Ray Hood	Beach MVCD, Florida
2018	Jessica Dieckmann	County of San Diego VCP, California
	James Wynn	Anastasia MVCD, Florida
	Stefan Sielsch	El Dorado County MVCD, California
	Kyle Yager	Hillsborough County MVCD, Florida
2019	James Binnall	North Shore MAD, Illinois
	Corey Boyer	Shasta MVCD, California
	David Delgado	Virgin Islands DH
	Aubrey Drummond	Virgin Islands DH
	Gerald Michael Hart	Indian River MCD, Florida
2020	Chad Kirkley	St. Tammany Parish MAD, Louisiana
	Trinidad Haro	Coachella Valley MVCD, California
2021	Reynaldo Morales	Puerto Rico VCU
	Rafael Saavedra-Hernandez	Puerto Rico VCU
	Marc Kensington	Coachella Valley MVCD, California
	Andrew Dewsnap	Salt Lake City MAD, Utah
2022	Bryan Ruiz	Delta MVCD, California
	Charles Rodriguez	Coachella MVCD, California
	Travis Edwards	Lee CMCD, Florida
	Greg Mercado	Greater LA CVCD, California
2023	Gonzalo Valadez	Coachella MVCD
	Cristhian Sánchez Rolón	Puerto Rico VCU
	Noemí Martínez-Tull	Puerto Rico VCU
	Yanet Chiong	Miami Dade County MCD

STUDENT PAPER COMPETITION AWARDS

The AMCA Student Competition was established in 1988 to recognize the outstanding student research paper presented at the annual meeting. Judging of oral presentations is based upon organization, delivery, clarity and effective use of visual aids. In 1991, a \$500 cash award was presented to the winner, and in 1998 the Hollandsworth Prize was established by the family of Gerald Hollandsworth to encourage student participation in the AMCA national meeting. There is a \$250 prize for honorable mention.

1989	Scott Willis	McNeese State U.	2009	Alexandra	University of Florida
1990	Andrea Brown	Peru State Coll.		Stephanie Larick*	University of Florida
1991	John Paul Mutebi	Notre Dame U.	201	Sarah Wheeler	University of California, Davis
1992	Rosmarie Kelly	U. Massachusetts		Kimmy Mains*	University of Kentucky
1993	Merry L. Holliday-	U. California, Davis		Holly Tuten*	Clemson University
1994	John E. Gimnig	U. California, Davis	2011	Logan Minter	University of Kentucky
	Alice Shaeffer*	U. Mainz, Germany		Kristen Meckel-	San Diego County Vector Control
1995	Glen Scoles	Notre Dame U.	201	Jerome Schleier	Montana State University
	Jittawadee Rochaeroen*	U. California, Riverside		Elizabeth Andrews*	University of Kentucky
1996	Esther Chow Schaeffer	U. Maryland		Jennifer Gordon*	University of Kentucky
1997	Lynn Cooper	U. Maryland		Joseph Iberg*	University of Georgia
1998	C. Roxanne Rutledge	Louisiana State U.	2013	Brian Johnson	Rutgers University
	Emmalee Kennedy*	U. Illinois		Andrea Egizi	Rutgers University
	Timothy Schaub*	U. Illinois		Brittany Nelms	U. California, Davis - CVEC
1999	Laura Harrington	U. Massachusetts	2014	James Ricci**	University of California
	Adam S. Jones*	U. Massachusetts		Eva Bickner***	University of Florida
	Hillary Reno*	U. Illinois		Allison Gardner***	U of IL Urbana - Champaign
2000	Jason L. Rasgon	U. California, Davis	2015	Maria Carrasquilla**	University of Florida
	Hope Q. Liu*	Virginia Polytechnic		Casey Parker***	University of Florida
2001	No competition		201	Sydney Crawley***	University of Kentucky
2002	Laura B. Goddard	U. California, Davis		Lin Zhu***	University of Miami
	Sharon L. Minnick*	U. California, Davis		Cassandra Urquhart**	University of Tennessee
	Margaret Sherriffs*	Yale U.	2017	Adena Why**	University of California

STUDENT PAPER COMPETITION AWARDS

2003	Sarah Yaremych	U. Illinois	Evlyn Pless **	University of California
	Laura Goddard*	U. California	Edmund Norris***	Iowa State University
	Jason L. Rasgon*	U. California, Davis	Annie Rich***	University of Georgia
2004	Gregory M. Williams	U. Delaware	Katelyn Haydett***	University of Georgia
	Stephen Aspen*	Colorado State U.	Jay Brown*	University of Georgia
	Christian Kaufmann*	U. Zurich	Christopher Bibbs*	Anastasia Mosquito Control Dist.
2005	Wesley Rubio	San Diego State U.	Shiloh Judd**	Louisiana State University
	Whitney Qualls*	Auburn University	Casey Parker*	University of Florida
	Rebecca Trout*	University of Kentucky	Ed Norris**	University of Florida
2006	Robert D. Anderson	University of	Meredith Beaulieu***	North Carolina State University
	Linda O'Connor**	University of	Raji Joshua***	Florida International University
	Joshua R. Ogawa*	Oregon State	Christopher Bibbs***	Anastasia Mosquito Control District
	Matthew Eaton*	Concordia College	Timothy McNamara*	Louisiana State University
	Linda M. Styer*	U. California, Davis	Corey Day**	University of Tennessee Knoxville
2007	Jennifer Armistead	University of Florida	Lindsay Baxter***	Cornell University
	Robert D. Anderson*	University of	Bob Aldridge***	USDA-ARS-CMAVE
	Thomas M. Mascari*	Louisiana State U.	Olayinka David***	Florida International University
2008	Jerome Schleier	Montana State	Kristina Lopez**	University of Wisconsin - Madison
	Christopher Barker*	U. California, Davis	Nicole Foley	Cornell University
	Lisa Reimer*	U. California, Davis	Kristin Sloyer***	University of Florida
			Antonio Alvarado***	Cornell University

* \$500 cash award presented to winner ** Gerald Hollandsworth Prize *** Honorable mention

AMCA OFFICERS, EXECUTIVE DIRECTORS AND EDITORS

AMCA PRESIDENTS

1935-1939	Thomas J. Headlee*	1968-1969	Thomas D. Mulhern	1995-1996	John D. Edman
1939-1940	Christian T. Williams*	1969-1970	George T. Carmichael	1996-1997	Robert J. Novak
1940-1942	Louis A. Stearns*	1970-1971	Albert W. Buzicky	1997-1998	Gary G. Clark
1942-1944	Robert C. Botsford*	1971-1972	Andrew J. Rogers	1998-1999	Dan L. Ariaz
1944-1945	Robert L. Vannote	1972-1973	Glen C. Collett	1999-2000	William J. Zawicki
1945-1946	Perry W. Ruth	1973-1974	Kenneth L. Knight	2000-2001	David A. Dame
1946-1947	Harry H. Stage	1974-1975	Robert M. Altman	2001-2002	Sammie L. Dickson
1947-1949	H. Duke Peters	1975-1976	Harold C. Chapman	2002-2003	David A. Brown
1949-1950	Harold F. Gray	1976-1977	D. Bruce Francy	2003-2004	Fred W. Knapp
1950-1951	Lester W. Smith	1977-1978	Lewis T. Nielsen	2004-2005	Roger S. Nasci
1951-1952	Don M. Rees	1978-1979	Paul J. Hunt	2005-2006	William R. Opp
1952-1953	Cecil R. Twinn	1979-1980	Glen M. Stokes	2006-2007	Joseph F. Sanzone
1953-1954	Fred C. Bishopp	1980-1981	Robert K. Washino	2007-2008	Gene R. Payne
1954-1955	Roland E. Dorer	1981-1982	Claude H. Schmidt	2008-2009	Major S. Dhillon
1955-1956	Richard F. Peters	1982-1983	Richard C. Axtell	2009-2010	Doug Carlson
1956-1957	Fred L. Stutz	1983-1984	Jimmy K. Olson	2010-2011	Janet McAllister
1957-1958	Arthur W. Lindquist	1984-1985	Gilbert L. Challet	2011-2012	William H. Meredith
1958-1959	John M. Hirst	1985-1986	T. Oscar Fultz	2012-2013	Thomas R. Wilmot
1959-1960	Archie D. Hess	1986-1987	Donald J. Sutherland	2013-2014	Roxanne Connelly
1960-1961	Daniel M. Jobbins	1987-1988	George B. Craig, Jr.	2014-2015	Steve Mulligan
1961-1962	William E. Bickley	1988-1989	Bruce F. Eldridge	2015-2016	Ken Linthicum
1962-1963	Arthur W. Geib	1989-1990	Judy A. Hansen	2016-2017	Stan Cope
1963-1964	Don W. Micks	1990-1991	Robert C. Sjogren	2017-2018	T. Wayne Gale
1964-1965	John A. Mulrennan,	1991-1992	Matthew Yates	2018-2019	William Walton
1965-1966	Anthony W. A. Brown	1992-1993	Cyrus R. Lesser	2019-2020	Jason Kinley
1966-1967	Jay E. Graham	1993-1994	John A. Mulrennan, Jr.	2020-2021	Ary Faraji
1967-1968	Harry D. Pratt	1994-1995	Chester G. Moore	2021-2022	Mark Breidenbaugh
				2022-2023	Dennis Walette

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AMCA TREASURERS

1935-1943	Thomas D. Mulhern *	1994-2000	Charles T. Palmisano
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1944-1950	Thomas D. Mulhern	2000-2011	Allan D. Inman
1950-1953	Roland E. Dorer	2011-present	Gary Hatch
1954-1964	Lester W. Smith		
1965-1979	William D. Murray		
1980-1985	James R. Caton		
1985-1986	Douglas C. White		
1986-1988	C. Lamar Meek		
1989-1994	John S. Billodeaux	* - Eastern Association of Mosquito Control Workers	

SECRETARY, EXECUTIVE SECRETARY, EXECUTIVE DIRECTOR

1935-1943	Thomas D. Mulhern*	Secretary	1992-1993	Harold C. Chapman	Executive Director
1944-1950	Thomas D. Mulhern	Secretary	1993-1994	Lucas G. Terracina	Acting Executive Dir.
1950-1952	Thomas D. Mulhern	Executive Secretary	1994-1995	Robert T. Graham	Executive Director
1953-1973	Theodore G. Raley	Executive Secretary	2006-2015	Sarah B. Gazi	Executive Director
1973	Theodore G. Raley	Executive Director	2015-2016	Lori Jensen	Executive Director
1974-1978	Thomas D. Mulhern	Executive Director	2016-2017	Bill Schankel	Executive Director
1979-1980	William D. Murray	Executive Director	2017-2019	Heather Gosciniak	Executive Director
1980-1985	Thomas D. Mulhern	Executive Director	2019-2020	David Butler	Executive Director
1985-1986	James R Caton	Interim Executive	2020 -present	Megan MacNee	Executive Director
1986-1991	Harold C. Chapman	Executive Director			
1991	Lucas G. Terracina	Acting Executive Dir.			
1992	Mark Vinsand	Executive Director			

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BUSINESS MANAGER

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1999-2000	Marlene Comeaux
2000-2001	Robertamarie Kiley
2001-2004	Martin. S. Chomsky
2004-2006	Sarah B. Gazi

TECHNICAL ADVISOR

2000-2020	Joseph M. Conlon
2020-present	David Brown

EDITORS OF *JOURNAL OF AMCA**

1941	Edited by the Publications Committee, Lester W. Smith, Chair [†]
1942-1943	Edited by the Publications Committee, Ralph W. Vanderwerker, Chair [†]
1944	Edited by the Publications Committee, J. T. Hart, Chair
1944-1948	Robert D. Glasgow
1949-1973	Donald L. Collins
1973-1981	William E. Bickley
1981-1996	Ronald A. Ward
1996-1998	Robert K. Washino
1999-2003	Bruce F. Eldridge
2004-2006	Kenneth J. Linthicum
2007- present	Lal S. Mian

* - *Mosquito News* became the *Journal* of AMCA in 1985

[†] - Publication of the Eastern Association of Mosquito Control Workers

[‡] - Volume 4, Number 1, was edited by the Publications Committee; subsequent volumes had a single editor

EDITORS OF *MOSQUITO SYSTEMATICS**

1969-1979	Kenneth L. Knight
1979-1992	Lewis T. Nielsen
1992-1993	Lewis T. Nielsen & Ralph E. Harbach, co-editors
1993-1995 [†]	Thomas J. Zavortink, editor, & Lewis T. Nielsen, editor emeritus

* - Prior to 1973 *Mosquito Systematics* was named *Mosquito Systematics Newsletter*

[†] - In 1995 this publication was discontinued