

ABSTRACT



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AMCA

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Plenary Session

PL-01 In Memoriam of Gary G. Clark (March 18, 1945-October 27, 2019): family man, scientist, and compassionate human being.

Dan Kline, dan.kline@usda.gov, Kenneth Linthicum

This presentation is to honor Gary G. Clark. Gary was born to Champ and Louise (Donoho) Clark on March 18, 1945, in San Diego, California, and was raised in Marion, Illinois, where he met his wife Carol Shillinger whom he married in 1967. Known for his sense of humor, Gary often jokingly referred to Carol as his first wife. They were married 52 years and raised three children together. Gary graduated from Southern Illinois University where he received a bachelor's degree in zoology. He earned a master's in wildlife biology and a doctorate in medical/veterinary entomology from Colorado State University. During his postdoctoral studies at the UCLA School of Public Health he conducted mosquito and virus field research in the low deserts of Riverside and Imperial Counties, California with world renowned arbovirologist Telford Work. This arduous research under the supervision of Dr. Work helped hone his scientific skills and especially his keen sense of humor which he retained throughout his illustrious career. He was highly regarded in scientific circles and was specifically known around the world in his field of arbovirology. He held many professional several positions during his illustrious career, including serving as Assistant Division Chief and Laboratory Manager at the Illinois Department of Public Health. He served as a U.S. Army Medical Service Corps Officer while conducting entomology and virus lab research at the high containment laboratories of USAMRIID at Fort Detrick, Maryland and field research in the Great Pocomoke-Cypress Swamp on the Eastern Shore of Maryland and Virginia. He subsequently transferred to the United States Public Health Service (USPHS) and eventually served for many years as entomologist and subsequently as Chief, Dengue Branch, Centers for Disease Control and Prevention in San Juan, Puerto Rico. After retiring from the USPHS at the rank of Captain he served as Research Leader of the Mosquito and Fly Research Unit at the U.S. Department of Agriculture Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida leading the Deployed Warfighter Protection Research program in the Unit. He was a member of and served a term as President of the American Mosquito Control Association (AMCA) and received its most prestigious award, the Medal of Honor. Additionally, he received a Presidential Citation and the Meritorious Service Medal from the AMCA, and served as Regional Director for the Latin American-Caribbean Region, member of the Editorial Board, and as a member of the Bylaws, International, Nominations, Awards, Publications and Central Office Committees of AMCA, an impressive and likely unequalled record in the history of the Association.

Within the AMCA and of special importance to him he founded, led, and published symposia review for the Latin American group and was a co-moderator for the first Spanish speaking session at an AMCA meeting. Dr. Clark always presented in Spanish when he would give a talk to Spanish-speaking audiences. It has been reported that he often joked that he was a Latin American that God punished by sending him in a gringo body to a Spanish speaking geographic area to conduct his research because he behaved badly in his past life. Gary was behind the creation of 'National Mosquito Control Week.' During his career he held numerous consultative positions for multiple organizations, such as the Pan American Health Organization (PAHO), the World Health Organization (WHO), the Bill and Melinda Gates Foundation and served as president of the San Juan (Puerto Rico) Rotary Club. It has been accurately written that, " Gary was a caring man known for his quick wit, infectious smile, and positive

attitude. He knew that, although we all come from different backgrounds and have different life experiences, everyone appreciates a smile or a funny anecdote as we cross paths in this world. He loved all sports, travelling and experiencing new cultures, and most of all spending time with his family". He will always be fondly remembered for his scientific achievements, support, humor and love of sports, especially the St. Louis Cardinals.

PL-02 Planetary Health: Considerations, Consequences and Opportunities for Vector Biology and Vector Control

Lyric Bartholomey, Lyric.bartholomey@wisc.edu

Many of us are familiar with the One Health concept, where human, animal and plant health are interconnected and demand interdisciplinary collaborations to achieve health and well-being for this triad. The scholarship of vector biology and practice of vector control are a poster child of One Health ideals. Planetary Health challenges us to mitigate and respond to threats to human health and well-being, with a particular eye on the consequences of anthropogenic influences on natural resources and biodiversity. Planetary Health proponents call for community-centered, situation-specific, and interdisciplinary approaches to human health threats. We can readily argue that professionals in our field have always been ahead of the game in understanding the interconnections of people, vertebrate animals, and the arthropods that feed on them, and that the AMCA has long fostered multidisciplinary interactions and projects focused on mitigating threats to public health. But we would be remiss in not seizing opportunities to take a leadership role in defining Planetary Health practices, to seek new connections and funding opportunities, and to learn from the Planetary Health movement.

Oral Presentation Abstracts

Student Competition I

6 Lengthy development times of Florida Culicoides influences density estimates of larval sampling methods

Theodore Black, th.black1@gmail.com, Nathan D Burkett-Cadena

We investigated the comparability of larval sampling techniques (emergence traps and laboratory rearing) for putative Culicoides EHDV vectors. Soil samples were collected adjacent to a large emergence trap and from underneath that trap's footprint. Soil samples were monitored under laboratory conditions for 11-13 weeks. *C. stellifer* displayed protracted emergence up to 13 weeks. The importance of time for estimates of Culicoides density for removed substrate sampling is significant both from a methodological and biological perspective.

7 Barrier screens and ramp traps: an evaluation of two unconventional methods for surveillance of Eastern equine encephalitis bridge vectors

Jamie Mangan, jlm566@cornell.edu, Linda Kalunga, Alex Ciota

We investigated Eastern equine encephalitis (EEE) bridge vectors in Virginia by comparing four collection methods: resting boxes, vegetation aspiration, ramp traps, and barrier screens. We evaluated the number and diversity of mosquito species collected and effort required. Over 20 species were captured during the 2021 study period. We conclude that ramp traps and barrier

screens can enhance mosquito collection diversity beyond standard resting boxes and CDC traps, thus enhancing vector biology research and control programs.

8 **Population Genetics among *Rhipicephalus sanguineus* ticks in the lower Rio Grande Valley**

Bianca Guerra, bianca.l.guerra01@utrgv.edu, Christopher Vitek, Erin Schuenzel, Rupesh Kariyat

Rhipicephalus sanguineus, the brown dog tick, is one of the most common tick species in the lower Rio Grande Valley. Their preferred host live in close proximity to humans and this species may serve as a reservoir hosts for pathogens that affect both human and other domestic animals. This study explores the genetic variation among different populations in the lower Rio Grande Valley by conducting a sequence analysis of 12s RNA mitochondrial gene where tropical and temperate sub-species will be determined. This species has both tropical and temperate lineages. These tick sub-species vary in vector competence, as they react different to pathogenic infection. The identification of sub-species in the Rio Grande valley may indicate relative risk of certain diseases. We hypothesis that we won't find the temperate subspecies in the Rio Grande Valley, given the average temperature and environmental surroundings in south Texas. Vectors were sampled from three different sites across a minimum of 15 miles between sites. Collections were conducted weekly for 4 months, using methods such as flagging and collecting off of domestic dogs. Q final total of 250 specimens were collected. The samples were then homogenized, DNA extracted using DNeasy blood and tissue kit, amplified, and analyzed by comparative sequencing of genetic strands between different populations. Results of the genetic analysis will be discussed. This data should help to identify which strain or strains of this tick are found in this region, which may help inform disease surveillance or control efforts.

9 **A habitat and risk model for disease vector *Aedes aegypti* (Diptera: Culicidae) in the Tampa Bay Area, Florida, USA.**

Connor Mapes, connormapes@usf.edu, Agne Prasauskas, Carl Boohene, Leonard Burns, Jason Stuck, Ryan Carney

To enable precision surveillance and control of important disease vector *Aedes aegypti*, we analyzed high-resolution aerial photography and three yr of CDC trap data from four counties in Tampa Bay, Florida using GIS. We combined MaxEnt ecological niche models and interpolated abundance data to quantify human risk and derive a novel "Vector Habitat Index." Results generated seasonal maps that are fine-grained (5m-8m) and actionable for the efficient and integrated vector management of this mosquito species.

10 **Investigation of Jamestown Canyon Virus Ecology in New Hampshire**

Joseph Poggi, jp2463@cornell.edu, Colin Conery, Denise Bolton, Rebecca Lovell, Abigail Mathewson, Laura Harrington, Marco Notarangelo

Jamestown Canyon (JCV) is a rare mosquito-borne virus with increasing prevalence in Eastern USA. Putative JCV vectors were collected in 2021 using a range of trapping methods. Twelve JCV positive pools from five species were detected in New Hampshire. *Aedes excrucians* infection rate per 1000 was highest (5.37, 95% CI 1.75 – 12.88) followed by *Aedes sticticus* and *Anopheles punctipennis*. Collectively, our study provides new insights into JCV transmission ecology in the Northeast region.

11 **Insecticide resistance in *Aedes* mosquitoes from 2018-2021 along the Texas-Mexico Border**

Thalia Rios, thalia.rios02@utrgv.edu, Heather Hernandez, Christopher Vitek

The first line of defense for vector-borne diseases is often controlling the vector population. Vector control is often becoming increasingly difficult due to insecticide resistance. It is vital for successful control efforts to determine if mosquito populations are becoming resistant to routinely used insecticides for control measures to be effective. We initiated a long-term project in 2018 to uncover patterns in insecticide resistance in cities along the Texas-Mexico border. We collected *Aedes* mosquito eggs from multiple cities along the border on a weekly basis. Collected eggs were hatched in the lab, identified for species as adults, and then tested for insecticide resistance using the CDC Bottle Bioassay to commonly used insecticides in the region: permethrin, deltamethrin, and sumethrin. . Permethrin was generally the most effective insecticide, while deltamethrin had the most limited efficacy in controlling mosquito populations. These results highlight the variable nature of insecticide resistance over time and the need for continual monitoring of resistance in mosquito population to ensure effective control of vectors.

12 **The New York State Tick Blitz: Harnessing Community Based Science to Understand Range Expansion of Ticks**

Nicole Foley, nf276@cornell.edu, Laura Harrington, Jody Gangloff-Kaufmann, Joellen Lampman, Siddhi Balamurali

We conducted a “Tick Blitz” in Southern New York State (NYS) to understand northward expansion of *Amblyomma americanum* and *Haemaphysalis longicornis*. Recruited volunteers were provided with tick biology and sampling education, followed by collection kits. Volunteers from 14 counties performed collections at 169 sites in mid-June 2021, capturing 3,760 ticks. Our results provide new information on *H. longicornis* and *A. americanum* established populations in NYS and highlight the power of citizen science for tick research.

Education/Public Relations I

13 **Measuring the effectiveness of the new Florida Department of Agriculture and Consumer Services (FDACS) public health online webinar series**

Marah Clark, Marah.Clark@fdacs.gov, Caitlin Gill, Jessica Ber, Johanna Welch, Bruce Nicely, Frieda Lamberg

In Florida, the primary mechanism for licensed public health mosquito control employees to earn continuing education units (CEUs) has been through in-person training. These options were unavailable in 2020 due to the ongoing COVID-19 pandemic, leaving many individuals without options to obtain required CEUs. In response, mosquito control specialists working for FDACS worked to develop online webinar classes using funds from the Center for Disease Control and Prevention’s Public Health Crisis Cooperative Agreement Grant for Hurricane Response. Because the online training format was new to the team, it was important to determine the effectiveness of both the training and the online platform. A survey document was created to be used as a part of the sessions to answer these questions. Evaluation topics focused on the overall effectiveness, what knowledge was

gained from the training, whether or not they would be able to use the information as a part of their mosquito control work, what they liked or did not like, and even asked for suggestions for future discussion topics. The survey tool was deployed through the online application as an email to the registered participants. Overall, there were 25 sessions, 865 attendees, almost a 49% survey response rate, and an overall effectiveness rate of 3.60 out of 4. The information obtained is used to improve training and develop classes pertinent to the needs of Florida's widely varying mosquito control programs. Due to the positive feedback from attendees and the current needs expressed by mosquito control program managers, additional classes have been created and offered beyond the initial grant period.

- 14 **Western Gulf Center of Excellence Outreach Education Update**
Sonja Swiger, slswiger@ag.tamu.edu, Scott Weaver, Phillip Kaufman

The Western Gulf Center of Vector Borne Diseases (WGCVBD) is one of five Center of Excellences (CoE) funded for a five year term to increase education and knowledge of vectors. One of the tasks taken on by the WGCVBD was to increase outreach education in Texas and neighboring states to municipality/city/county/state and governmental employees. An overview of the educational classes developed and implemented will be provided with for the five year period of the CoE.

- 15 **BEACONS: An invasive mosquito surveillance, communication, and research working group for the southern United States**
Dan Killingsworth, dan.killingsworth@gmail.com, Bryan Giordano, YOOSOOK LEE, LINDSAY CAMPBELL, Michael T. Riles, Benjamin Allen

Over the past decade, the southern region of the United States has observed a drastic increase in the number of invasive mosquito species introductions and range-expansions. Nevertheless, no regional or national contingency plans are established to monitor, mitigate, or stop these introductions. The mosquito BEACONS (Biodiversity Enhancement And Control Of Non-native Species) working group established in March of 2021 contains members from academia, public health, private pest control, and mosquito control programs. This novel partnership is designed to help combat mosquito invasions, develop integrative pest management research proposals, and increase the capacity for surveillance activities in the southern United States. Here we present results from a survey (IRB202101286) completed by mosquito control programs across the southern states including Mississippi, Louisiana, Georgia, Florida, North Carolina, and South Carolina. This baseline data is necessary to identify gaps in knowledge and surveillance pertaining to invasive mosquito species. This critical information will be used to create professional development and training modules which address stakeholder needs and emerging issues. The BEACONS program was funded by a grant provided through the Southern Integrated Pest Management Center.

- 16 **Positive outcomes from a pivot to virtual environmental education during the pandemic**
Andrea Miller, amiller@lcmcd.org, Eric Jackson

The pandemic has created many unforeseen circumstances across all professions but especially in the field of education. During these uniquely challenging times, it is what we do with the situation that truly matters. In this session, discover how the robust Lee County Mosquito Education (LCME) program used the trials and tribulations of the pandemic as a springboard into virtual education

that resulted in a record setting program reach of over 20,000 students during the 2020-21 school year. Learn more about the virtual activities, presentations and labs that were developed to maintain instructional continuity while also enhancing the LCME program.

- 17 **Educating our community one stop at a time: Hillsborough County’s mobile laboratory**
Alexa Patrizio, patrizioa@HillsboroughCounty.org, Ronald Montgomery, Mary Geesey

The origin of Hillsborough County’s mobile public education and outreach laboratory was as an extension of the mosquito fish giveaway program. It all began with our mosquito fish truck, which debuted at DoDD in 2019. The increase in Citizen interest resulted in program expansion of the mosquito fish giveaway events – hence, the “Public Education and Outreach Mobile Lab” trailer was born. Prior to the mobile lab’s debut at DoDD in 2020, the mobile lab accompanied the mosquito fish truck to the giveaway events every other Saturday during the summer of 2019. The mobile public education lab showcases the mosquito life-cycle and MMS Operations through bold and colorful graphics and technology, such as a video screen set up with a microscope. Hillsborough County’s mobile laboratory has proven wildly popular across all generations. We will review the costs associated with the build and how we altered the interior to accommodate our design vision.

Keywords: Mobile lab, mosquito life-cycle, public education and outreach

- 18 **Leverage the power of NASA science to promote mosquito vector education and source reduction in your district**
Russanne Low, rusty_low@strategies.org

The GLOBE Observer Mosquito Habitat Mapper is a free mobile app ready for use in education and outreach programs in your district. Funded by NASA, the app enables citizen scientists to report when and where mosquito larvae and larval habitats are observed in and around their communities. These data complement remotely-sensed environmental data from space that are routinely used in the development of predictive models of mosquito vector-borne disease.

App users are asked to upload photos of the larval habitats and larvae they encounter. An optional step enables users to determine if their specimen belongs to one of three medically important genera, *Aedes aegypti* or *Aedes albopictus*, using a built-in pictorial key.

In addition to obtaining scientific data, the app was designed to support local larval habitat mitigation efforts. In the last step, users are asked to “tip, toss, or cover” potential container habitats, and the app tallies user reports of source reduction. These data can be used to provide metrics of effectiveness of community-based mitigation and outreach programs. A team function enables counties and districts to access data collected by citizen scientists locally through a data dashboard.

You can leverage the power of NASA science to excite and engage community members in mosquito vector education and outreach. Citizen science interest and engagement is supported by periodic data challenges and social media campaigns. The GLOBE Observer project offers a wide range of freely available educational resources you can use in education and outreach programming: for selected examples, see <https://observer.globe.gov/mosquito-challenge>.

- 19 **Takeaways gained from residents with an urban residential mosquito complaint**
Katherine Ramirez, kramirez@mosquitobuzz.net, Jodi Holeman, Steve Mulligan

Prior to 2013, the Consolidated Mosquito Abatement District did not have a daytime-biting mosquito established in its urban residential service areas. In June 2013, *Aedes aegypti* was detected in a Clovis, CA neighborhood. Within five years, *Ae. aegypti* expanded its range to encompass all urban cities and communities in the District's 1,058 square mile service area. The invasion and spread of this mosquito resulted in dramatic increases in the number of requests for service from the public directly related to the high biting pressure experienced by residents in urban areas. In response, over the last seven years, our key mosquito prevention messages of screening every yard drain and dumping every source have been promoted at every site inspection. However, our urban residents continue to call in to voice the same concerns about mosquitoes despite clear communication on a prior site inspection. The District began to question the communication provided during urban site inspections. We wanted to know if residents were dumping sources and screening their yard drains, as recommended, following a site inspection. To answer these questions, a seven-question survey for residents was developed. The survey was sent out in November 2020 to each resident who had an urban residential mosquito complaint and provided an email address. There were 825 surveys sent out with a 35% response rate. The takeaways gained from the survey responses and how they impacted the approach for 2021 urban residential service requests will be discussed.

Mosquito Lightning Symposium

- 20 **MCU, the deployment of the first Monkey Control Unit within a Mosquito Control District**
Ary Faraji, ary@slcmad.org

Mosquito control has generally been conducted by humans and associated biological control agents for the suppression of mosquitoes and mosquito-borne pathogens. Often times, prime mosquito control habitats are cryptic and difficult to access. Herein we describe the theoretical deployment of the first Monkey Control Unit (MCU) within a Mosquito Control District. If policemen can use K9 units successfully, then there is no reason why an MCU could not be efficiently utilized by mosquito control inspectors.

- 21 **PIWI's big adventure: endogenous viral elements in vector arthropod genomes**
Dana Price, d.price@rutgers.edu, Miranda Barnes

The documentation of endogenous viral elements (EVEs) - virus-derived genetic information integrated into the genomes of a host and its descendants - has offered new insights into how arthropods such as mosquitos and ticks respond to viral infection via RNA-mediated silencing pathways, and their capacity to spread and maintain vector-borne viruses in the environment. Recent advances in genome sequencing have resulted in a wealth of highly contiguous tick genomes, and with them, the ability to identify and assess the provenance and shared ancestry of EVE elements in multiple lineages. Our analysis uses informatic approaches to analyze the available sequenced genomes of nine tick species for EVEs, with special reference to Non-retroviral Integrated RNA Virus (or NIRV) elements. Results highlight multiple independent acquisitions and of similar EVEs among congeneric hosts as well

as shared derived viral elements presumably acquired prior to the divergence of multiple genera. These results will inform current and future work regarding past associations with, and future vector potential of, arthropod vector species with regard to the viruses from which their “viral fossils” are derived.

22 **Sublethal effects of insecticides on phenotypic traits of mosquitoes and their ability to transmit arboviruses**

Barry Alto, bwalto@ufl.edu, Abdullah Alomar

Dengue virus is one of the most prevalent vector-borne viruses that causes life-threatening illness to humans worldwide. Development of new tools to control vector population has potential to reduce the burden of dengue virus. Toxic sugar bait (TSB) is a new form of vector control that targets the physiologically relevant behavior of sugar feeding of adults to deliver gut toxins. Studies on the effects of TSB ingestion on important aspects of vector biology, including vector competence for viruses are lacking. In this study, adult female *Aedes albopictus* longevity and vector competence for dengue virus were assessed following the ingestion of TSB formulated with spinosad as an oral toxin. Ingestion of TSB caused reduction on longevity of females. Higher dengue virus infection, disseminated infection and transmission rates were observed in females that ingested TSB compared to controls at each time point. We show that although ingestion of TSB reduced vector longevity, it can produce individuals with enhanced vector competence for dengue virus. Our results highlight the importance of evaluating the net impact of sublethal effects of insecticides on parameters of vectorial capacity in context of vector control efforts to reduce risk of transmission of vector-borne viruses.

24 **Considerations of morphologic observations of mosquito species from identifying complete samples in Panama City Beach, Florida, U.S.A.**

Michael T. Riles, michael@pcbeachmosquito.com

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

25 **The role of vector diversity in dog heartworm transmission**

Meredith Spence Beaulieu, meredithspence@gmail.com, Michael Reiskind

Mathematical models have long been used to investigate the dynamics of and important contributors to vector-borne disease transmission. Despite a plethora of vector-borne diseases being transmitted by an assembly of vectors rather than a single primary vector, models have scarcely addressed the effects of vector diversity on disease transmission. We developed a multi-vector compartmental model of dog heartworm disease, caused by the mosquito-borne nematode *Dirofilaria immitis*, to assess how changes in vector diversity impact transmission potential. Using laboratory generated parameters and mosquito community composition field data, we compared the R_0 of a diverse natural assemblage to that of a less diverse suburban assemblage. We also investigated the

effect of seasonality on heartworm transmission. We demonstrated a greater R_0 for the natural mosquito assemblage than that of the suburban assemblage, validating previous empirical observations of lower heartworm prevalence in suburban areas. Simulations suggest that vector diversity serves as a stabilizing force in the transmission of multi-vectorized pathogens, while species identity and community composition determine the magnitude of transmission.

26 **ADAPCO Vector Lab – Rated MPO**

Casey Crockett, ccrockett@myadapco.com, Emily Boothe, Katie Williams, Jason Trumbetta

In the vector control space, the application of adulticides and larvicides is utilized to control immature and adult mosquitoes. Throughout the US, this work is carried out by hundreds of licensed pesticide applicators working in the public health vector control sector. In addition to their application responsibilities, these individuals are required to maintain their applicator's license by earning Continuing Education Units (CEUs) on a regular basis. Prior to the COVID-19 pandemic, CEU credits could easily be earned through in-person annual meetings, training workshops, or locally hosted seminars. In a non-COVID year, acquiring the necessary credits was not an insurmountable feat, but with the complete absence of in-person training as a result of COVID-19 came the near complete-absence of potential to earn CEUs for many applicators. This educational void in the vector control industry gave rise to ADAPCO Vector Lab, a fully online platform for earning CEUs while taking courses specific to operational vector control. ADAPCO Vector Lab represents a new versatile and growing training resource, specific to vector control (rated MPO for mosquito professionals only).

27 **Lessons learned with UASS**

Jane Bonds, jasbonds@gmail.com

The application of vector control products is a crucial component of integrated vector management, a technique we are constantly refining. In pesticide application Unmanned Aerial Spray Systems (UASS) could fill in the gap between manned aircraft and ground equipment. They offer improved efficiency and a more responsible approach from an economical, ecological and human risk perspective. Our team of aerial application experts are developing the necessary protocols and equipment for standardized testing and optimization of UASS.

There are clearly advantages and disadvantages to the use of UASS in mosquito control, agriculture and forestry. A balanced approach to their use is needed now the excitement has died down a little bit. Lets talk about the lessons learned.

28 **Predation of the Cuban Treefrog (*Osteopilus septentrionalis*) by *Psorophora ciliata***

David Hoel, HOEL@LCMCD.ORG, Anne Kennedy, Kara Tyler-Julian

Psorophora ciliata is the largest of four *Psorophora* mosquitoes found in Lee County in Southwest Florida. It is often found breeding in with *Ps. columbiae*, the dark rice field mosquito, in the many open fields, pastures, and roadside ditches of Lee County. Recently, *Psorophora* larvae were found breeding in tire tracks in a new residential neighborhood. This neighborhood, still without houses, was until recently a cow pasture that produced tremendous numbers of *Ps. columbiae*. In developing this site, all trees, grass and shrubs were removed, the field was graded, and overlain with a crushed-shell material. In the interim, some grass and weeds have reestablished on this otherwise barren landscape.

Psorophora larvae were found in a flooded tire track with emergent vegetation. *Ps. columbiae* were plentiful in larval dips, and *Ps. ciliata* were quickly noted due to their large size, quadrate head, and predation on smaller larvae. While dipping to establish a larval density required before treatment, we found and photographed a *Ps. ciliata* larva with what we thought was *Ps. columbiae* in its mouthparts. Further examination revealed that its meal consisted of a tadpole, identified as a Cuban Treefrog (*Osteopilus septentrionalis*) according to online keys and from tadpoles collected and reared to the adult stage from like-occurring tadpoles collected from the same tire track. Although amphibian predation by *Ps. ciliata* is noted in the literature, this is the first instance we have seen of it through routine surveillance. The photographs show a fourth instar *Ps. ciliata* in the process of eating a Gosner stage 23-25 Cuban Treefrog tadpole.

29 **Covid a hurdle too high**

Herff Jones, hjones@iberiagov.net

Corona virus disease 2019 (COVID-19) from middle 2019, all of 2020 and most of 2021, plunged the world into the depths of a global pandemic. Sever acute respiratory syndrome corona virus variant 2 (SARS CoV2) brought the world to its knees. Global systems for commerce, economics, science, government, technology, banking, academics, health care and yes, mosquito control programs faced alterations due to the conditions set forth by this public health crisis. Control programs implemented protocols to provide maximize protection for staff, adjusted standard operating procedures to secure mission directives and balanced exposure versus obligation to program's citizenry. Throughout this crisis there have been ebbs and flows, mitigation, quarantine, vaccine, then a variant, a hurdle too high. Delta variant infections and exposures disseminated control programs' staff and reduced operational capacities requiring program administrators to improvise and adapt.

30 **Communicating vector management to the public: expect the unexpected!**

Marianyoly Ortiz, mortiz@prvectorcontrol.org, Grayson Brown, Julieanne Miranda-Bermúdez

In this era of social media and anti-science, communicating new advances in public health and vector management is not an easy task. How do you approach the public to create awareness, obtain their support and avoid negative media backlash? In Puerto Rico, public pressure has occasionally been strong enough to derail public health efforts and even to force politicians to resign. However, non-traditional approaches, like the release of millions of *Wolbachia* mosquitoes, were received with wide acceptance. Is there a secret to achieving public acceptance? Probably not, but there are several strategies that have proven to be successful and that had helped the Puerto Rico Vector Control Unit to implement new approaches on the island. In this presentation, we will provide a quick view of what we have faced and how have we prepared for the unexpected.

31 **West Nile Virus Explosion After Covid-19 Coverup**

Desiree Keeney, dkeeney@adacounty.id.gov

Looking at the aftermath of the global pandemic, COVID-19, Ada County Mosquito Abatement District started to pick up the pieces only to find the continued effects seep into the social, economic and day to day operations a year later. As the mosquito season started to ramp up along with record breaking heat degree days and drought, an explosion of West Nile virus (WNV) occurred and the residual effects of COVID-19 spun a local community into a social media spiral. This presentation will

take a quick look at the big picture of the worst WNV year since it first appeared and Idaho led the nation in WNV cases in 2006, community mistrust and acceptance and the social and political impacts that resulted.

32 **Can endectocides stop West Nile virus?**

Christopher Barker, cmbarker@ucdavis.edu

West Nile virus (WNV) is the most common cause of arboviral human disease in the U.S. in most years, and it continues to pose major challenges annually for mosquito control programs in most of the country. As a zoonotic arbovirus, WNV is maintained in enzootic cycles between bird-biting mosquitoes and various species of wild birds. The precision of WNV control programs is limited by the inability of conventional insecticides to selectively target WNV vector species and by concerns about exposure of humans to pesticides in urbanized areas. The endectocide, ivermectin, offers a potential alternative that could be fed to wild birds to kill the mosquitoes that feed upon them, thereby interrupting viral amplification in residential areas without the need to broadcast insecticides. This presentation will provide an update on a collaborative study combining laboratory and field research with modeling to determine the viability of ivermectin as a WNV control strategy.

33 **Will AI brick the entomologist?**

Eric Haas-Stapleton, Eric@mosquitoes.org, Pranathi Vemuri

Artificial intelligence (AI) is rapidly changing how data is analyzed and decisions are made. We developed AI algorithms that accurately discriminated mosquitoes from debris or other insects in images that were captured with a mobile phone. Once categorized as mosquitoes, the algorithm accurately categorized Culex mosquitoes to species. Notably, the algorithm flagged mosquito species that were not known to it for humans to evaluate. Vector control entomologists may be encouraged to build out their scope of work as AI becomes more broadly adopted to automate lab workflows.

34 **Mr. Mister: Rockin' the Aedes of the San Francisco Bay salt marshes**

Mark Wieland, MarkW@mosquitoes.org, Joseph Huston, Ryan Clausnitzer, Eric Haas-Stapleton

Turbine mist sprayers (TMS) are being rapidly adopted nationwide for applying larvicide to control peridomestic *Aedes aegypti*. Because TMS can loft large quantities of larvicide over relatively long distances, we examined its efficacy in a tidal marsh habitat for controlling *Aedes dorsalis*. Liquid Vectobac 12AS larvicide was applied at 1 pt / ac using a TMS with the output nozzle directed 5 – 15 degrees below horizontal at a ground speed of 10 mph from a levy that was elevated 3 – 6 ft above the tidal marsh where surface wind speed was 10.7 mph. Cards that change color when exposed to liquid were placed perpendicular to the path of the TMS showed the larvicide mist traveled up to 60 yd from the TMS and did not extend to 90 yd. Use of the TMS enabled a 4-fold increase in total acres treated during 2020 – 2021 relative to the prior 2 yr (3777 and 922 ac, respectively) without a concomitant increase in staff time. There was no difference in the number of high tide events that may have affected the number of *Ae. dorsalis* egg hatches. Notably, there was an 83% reduction in the quantity of *Ae. dorsalis* larvae at 5 d post treatment. Similarly, there was a 63% reduction in adult female *Ae. dorsalis* that were collected in EVS traps from nearby communities relative to the prior 2 yr. There were 2.3-fold fewer requests for service to address a mosquito problem from residents of cities that abut the tidal

marshes, suggesting the applications had a positive impact on these communities. TMS offer an attractive alternative to hand treatments in tidal marshes where the use of unmanned aircraft or all-terrain vehicles is prohibited by national wildlife refuge managers.

- 35 **Wrangling *Aedes sollicitans* (Diptera: Culicidae) in rural Manatee County, Florida**
Samantha Ramirez, s.ramirez@manateemosquito.com, Dwight Andress, Katie Williams,
Christopher Lesser

Aedes sollicitans (Walker) is one of the nuisance saltmarsh mosquitoes that also serves as a vector for Eastern equine encephalitis, Venezuelan equine encephalitis and *Dirofilaria immitis* (Leidy). In Manatee County this pest mosquito has historically only settled in the saline environment of the coastline. Yet, as this spring approached a new *Ae. sollicitans* habitat was discovered about 20 mi east of their typical conditions in a dairy farm where thousands of healthy larvae and the opportunistic adults were identified. This began an investigation in determining just how these mosquitoes got there and how stable the artificial environment was. To determine if this was a one-time occurrence, the salinity of the standing water and periodic rainwater throughout the fields was recorded for several weeks. In addition, larval samples were brought in for eventual adult resistance testing with our current rotation of adulticides. The owner confirmed his flood irrigation method to water his fields and adding salt to yield not only a nutritious grazing crop but also an unintentional mosquito habitat. While inland occurrences are not uncommon, the substantial population of *Ae. sollicitans* was remarkable and will be diligently monitored.

- 36 **Insect orientation takes flight with mosquitoes**
Neil Vickers, neil@slcmad.org

The behavioral mechanisms by which flying insects locate a remote resource using only invisible wind-borne cues such as odors have been the subject of significant scientific interest and study. The first experiments to explore these mechanisms in insects was published in 1940 by John S. Kennedy. His paper has enjoyed considerable and deserved longevity in the literature and forms the basis of our current understanding of how insects accomplish this remarkable feat. Interestingly, in his experiments, Kennedy used *Aedes aegypti* and, as such, the yellow-fever mosquito forms part of the historical foundation for the study of insect orientation behavior. In this lightning talk I will briefly revisit Kennedy's seminal paper and its legacy.

- 37 **Reduced calorie mosquito killer**
Greg White, greg@slcmad.org

The use of toxic baits to kill insects has been used for decades to reduce populations of many pests such as cockroaches, termites, and ants. This method was also used for mosquitoes decades ago by mixing sugar and malathion, but never gained widespread use. A number of more recent studies have shown the potential for using baits with toxicants to control mosquitoes. While looking into ways of increasing frass production in mosquitoes using sugar alcohols, we found that erythritol was promising as an ingestible insecticide. Multiple labs confirmed this using different species of mosquitoes. Erythritol is commonly used a low-calorie sweetener in food for human consumption and may be effective as toxicant in a mosquito bait.

38

One Florida mosquito control district's expansion and challenges

Adriane Rogers, arogers@pascomosquito.org

Pasco County, Florida is currently one of the most rapidly growing counties in the nation. To keep up with the human population increase and urban growth, the Pasco County Mosquito Control District (PCMCD) must adapt to the changing landscape and increase in customers served. The PCMCD has a long history of providing quality service to its residents and visitors alike and helping to maintain quality of life and vector-borne disease protection. Part of the much-needed growth has come in the form of land acquisition to build-out a new operating campus, increasing staff size, and adding new equipment and technologies to the integrated mosquito management program. Change is inevitable, but change can also be quite challenging. Flexibility and resourcefulness are necessary to grow a mosquito control program. And so is perseverance!

39

Hesperus arizonicus: A terrestrial rove beetle and underwater predator of mosquito larvae

Lawrence Reeves, lereeves@ufl.edu

Night has fallen on the Sonoran Desert. At the mouth of Carr Canyon in the Huachuca Mountains of southeastern Arizona, a tiny predator awakens: *Hesperus arizonicus*, a rove beetle, about 1.5 cm from the head to the apex of the abdomen. It perches itself at the edge of a water-holding tree hole in an oak, inhabited by the larvae of *Aedes burgeri*, *Aedes purpureipes*, *Orthopodomyia kummi*, and other mosquito species. In the Pantanal, the expansive tropical wetland south of the Amazon, there are jaguars that have learned to hunt caiman. There, the terrestrial cats stalk caiman from the banks of the waterway, diving in to attack and kill the largely aquatic crocodilians, dragging their prey back up onto land to be consumed. Back in the Sonoran Desert, the rove beetle, just like the caiman-hunting jaguars of the Pantanal, dives into the water of the tree hole. Cloaked in a bubble of air, the beetle crawls down the walls of the tree hole, underwater, exploring and hunting. The beetle stays submerged for more than a minute until it successfully captures a mosquito larva. Holding the writhing larva in its jaws, the beetle drags it up the side of the tree hole to the surface, where it is consumed. Brought back to the lab and given access to mosquito larvae nightly, we found that *Hesperus arizonicus* can consume approximately 20 fourth instar larvae per night, a rate similar to fourth instar *Toxorhynchites* larvae. The desert has many secrets. This one, uncovered during the monsoon season of 2021, one of the wettest on record, demonstrates that in addition to other aquatic coleopteran predators of larval mosquitoes, there are also terrestrial beetles that venture underwater to hunt mosquito prey.

Student Competition II

40

Time of day variation in permethrin susceptibility and metabolic gene expression in *Aedes aegypti*

Sierra Schluep, ssschluep@ufl.edu, Eva Buckner

Using the active ingredient permethrin, we calculated the LD50 of an *Aedes aegypti* population exhibiting metabolic resistance conferred by three major classes of detoxification enzymes and a susceptible population at 6 time points throughout the light: dark cycle. Both populations were most resistant in the scotophase. However, genes belonging to each class exhibited increased

expression during the scotophase only for the resistant population. Metabolic resistance may correlate with time of day in *Ae. aegypti*.

41 **Insecticide resistance of West Nile virus vectors in the Chicago suburbs**

Kristina Lopez, kalopez@wisc.edu, PATRICK IRWIN, Daniel Bartlett, Susan Paskewitz, Lyric Bartholomay

Minimal reduction in mosquito abundance post adulticide application prompted product efficacy evaluations. We conducted CDC bottle bioassays, topical applications, and caged field trials on susceptible *Culex pipiens*, wild *Cx. pipiens*, and wild *Cx. restuans* collected from six study sites in the northwest suburbs of Chicago, IL. Results showed wild *Cx. pipiens* were resistant to all products tested, where wild *Cx. restuans* were susceptible.

42 **Can Allethrin-Based Spatial Repellents Protect White-tailed Deer from Mosquitoes and Biting Midges?**

Daniel Tuzzolino, dtuzzolino1@ufl.edu, Nathan D Burkett-Cadena

The utility of spatial repellents for protecting livestock has not been thoroughly explored. We evaluated the effectiveness of Allethrin-based spatial repellents for protecting white-tailed deer from host seeking mosquitoes and *Culicoides* at a deer farm in northern Florida. CDC light traps equipped with black light emitting LED diodes were operated ~1 m away from feeders with and without spatial repellents during the summer. Preliminary data suggest fewer blood-feeding Diptera were captured with Allethrin-based spatial repellents.

43 **Habitat complexity affects damselfly nymph (*Enallagma*) predation on *Culex pipiens***

Jaclyn Everly, jeverl1@ilstu.edu

Culex pipiens develops in ephemeral pools that can undergo significant structural changes as rain accumulates and evaporates. Increasing water volume creates more space between vegetation and the water surface (forming a more open aquatic habitat), while decreasing water volume reduces the ratio of open water to vegetation (forming a comparatively less open and more structurally complex aquatic habitat). In the midwest, ephemeral pools can contain a variety of invertebrates including *C. pipiens* mosquito larvae and predators like damselfly nymphs. To better understand the role of locally abundant *Enallagma* damselfly nymphs as predators in *C. pipiens* habitats, we performed a functional response experiment in which habitat complexity and prey availability were manipulated. One damselfly nymph was presented with either 10, 20, 30, 60, or 120 second instar mosquito larvae in either a simple or complex habitat. Simple habitats consisted of deli cups containing water and a 11 cm applicator stick, while the complex habitats included ten strips of grid wire screening attached to the applicator stick pointing upward and outward, simulating the branching structure of common aquatic vegetation. We hypothesized that damselfly nymph predation would increase with prey availability up until the point of nymph satiety, thus exhibiting a hyperbolic, Type II functional response. Therefore, we predicted highest predation around intermediate to high prey densities (e.g., prey density = 60). We also hypothesized that environmental complexity would decrease damselfly nymph predation via decreased encounters with prey. We predicted greater estimated attack rate in the simple habitats than in the complex habitats.

- 44 **Effectiveness of catch basin larvicide applications on *Culex pipiens* and *Cx. restuans* in the greater Milwaukee, WI area**
Haley Johnson, hejohnson4@wisc.edu, Justin Harbison, Mitchell Kirsch, Mark Clifton, Susan Paskewitz, Lyric Bartholomay

We evaluated the impact of catch basin larvicide applications on adult *Culex* abundance at an operational scale in the Milwaukee metropolitan area. A stepped-wedge design allowed us to assess the effect of VectoLex[®] FG applications in four 1mi² sites (800-1000 basins each) on *Cx. pipiens* and *Cx. restuans* numbers. Treatment effects were measured using basin backchecks, and CDC light and gravid traps. Despite successful larval suppression, no significant impact was observed for adult abundance.

- 45 **Larvicidal activity of three aromatic plant extracts on *Anopheles gambiae* s.l. and their acute oral toxicity**
Chi Fru, tchampofru@gmail.com, Metoh Njuabe, Herman Ambene

As an alternative strategy to control mosquito vectors, we accessed the larvicidal activity of *Momordica foetida*, *Gnidia glauca* and *Vepris soyauxii*, extracts on *An. gambiae* s.l. larvae and evaluated their acute oral toxicity. The extracts demonstrated larvicidal properties, where *V. soyauxii* methanolic extract showed the highest larvicidal activity when exposed to third instar larvae. The absence of toxic symptoms on Wistar rats makes them possible leads in the search for new eco-friendly and biodegradable larvicides.

- 46 **Efficacy of VectoMax[®]G larvicide for the control of mosquito densities and malaria transmission in the city of Yaoundé, Cameroon**
Nina Ghislaine Yensii, ninaghislaine03@gmail.com, Patricia Doumbe-Belisse, Carmene Sandra Ngadeu, Abdou Talipouo, Landry Djamouko-Djonkam, Ngangue Siewe Idriss, Metoh Njuabe, Christophe Antonio-Nkondjio

This study assessed the efficacy of VectoMax[®]G larvicide for reducing mosquito densities and malaria transmission in Yaoundé, as other control methods have challenges of drug and insecticide resistance. After treating some clusters with VectoMax[®]G and some left as control, mosquitoes collected from both treated and control clusters were analyzed. A high reduction rate recorded in mosquito densities and infection rates in treated clusters showed the efficacy of VectoMax[®]G in reducing mosquito densities and malaria transmission.

Education/Public Relations II

- 48 **Standing up for mosquito control in difficult times of public perception and justifying your job**
Miranda Tressler, MTressler@volusia.org, Tim Machardy

Those who work directly in mosquito control operations are familiar with the importance their roles play in protecting the public. Often we conduct operations in response to arbovirus activity without the direct knowledge of the public and sometimes in opposition of public

opinion. Mosquito control serves in the shadows, protecting their residents day and night during the harshest times of the year.

How do you respond when a vocal citizen spreads rumors of environmental misconduct? How do you continue to stay positive and stand up for your programs in these times when citizens seek to dissolve your chemical budgets and promote the funding be used for bat boxes? How do you act when a citizen intent on closing down mosquito control tells your county councilmembers that you should find a new career?

Volusia County Mosquito Control strives to provide our residents with science based facts including the details of all of the components of our integrated pest management program. We open the doors for conversations with any resident who is interested in learning about how we make daily treatment decisions. We stand up for our employees and we do what's best for our community to protect public health.

49 **Adventures in communicating about vector-borne diseases**
Jonathan Day, dnellson@comcast.net

Consumers and community leaders are often confused by the science behind vector-borne disease risk assessment and the resulting vector control strategies. The accurate communication of disease risk, the value of vector control measures, and how individuals can protect themselves is a critical part of managing a vector-borne disease outbreak.

Predicting vector-borne disease transmission risk requires a real-time understanding of vector populations along with an understanding of the interaction of vectors with amplification and reservoir host populations. In addition to tracking vector and amplification host populations, an understanding is needed of how environmental factors (rainfall, drought, and temperature) drive the interactions of vectors, pathogens, and vertebrate hosts. Sometimes the environmental signatures driving a vector-borne disease outbreak are clear, sometimes they are not.

The communication of vector-borne disease transmission risk is difficult. Often, it is well after an outbreak begins that the warning signs become clear. We constantly straddle the thin line of “crying wolf” when there is little risk of a vector-borne disease outbreak, and “being blindsided” by an epidemic that showed few early signs of development. In this talk I will share examples of communication devices that show promise for the accurate and timely communication of real-time vector-borne disease risk. I will also discuss the efficacy of public health measures and personal protection strategies that can be used to mitigate an outbreak of vector-borne disease.

52 **A New Approach: Bringing Virtual Mosquito Control to Homeowner's Associations**
Diana Garcia, dgarcia@glacvcd.org

With over one million parcels and an established invasive *Aedes* population spreading to new communities each year, it has been increasingly challenging for inspectors in the Greater Los Angeles County Vector Control District (GLACVCD / District) to conduct individual property inspections during mosquito season. GLACVCD's Operations and Communications teams work together to identify Homeowners Associations (HOAs) with common breeding sources to provide a virtual presentation rather than having a technician inspect each property. This analysis showcases operation labor costs in one day and compares the labor cost to a Community Liaison coordinating and conducting the

presentation. By conducting these virtual presentations, results show a decrease in Operation labor costs, an increase in community engagement, and the opportunity to build a relationship with the HOA.

53 **Review of your IPM plan can refocus your organization**

Mark Smith, mmcd_mes@mmcd.org

The Metropolitan Mosquito Control District has reviewed their Integrated Pest Management plans to assist in the revitalization of the team-based organization. This critical review has led to new ideas, changes in team structure, training opportunities, and refocused staff on the District's mission.

54 **Social Media Internship Program Influences Gen Z in Placer County**

Meagan Luevano, Meaganl@placermosquito.org

After over a year of a solely digital outreach strategy due to COVID-19, Placer Mosquito hired a social media intern to help boost engagement on TikTok and Instagram to continue its digital outreach momentum during 2021 West Nile virus season. While the District maintains established profiles on both TikTok and Instagram, both platforms experience low engagement analytics. District staff felt strongly that hiring an intern from a local high school would provide an introduction into the public health field and an opportunity for the intern to learn professional communication and public relations skills in a real work environment. The internship also allowed the District to create meaningful content to reach Gen Z while also meeting the District's strategic goal to spread awareness and educate the Placer County public about the risks from vectors and vector-borne diseases.

The AMCA Research Fund: Coordinating the Efforts of Researchers and the

AMCA Community for Greater Impact Symposium I

55 **A brief history of the AMCA Research Fund**

Jennifer Henke, JHenke@cvmosquito.org, Edmund Norris

The AMCA Research Fund has funded two projects annually since 2017. In this time, there has been excellent research funded which is of interest to mosquito control professionals. This symposium is meant to fulfill the requirements that the most recent recipients give a presentation. This presentation will highlight how the AMCA RF was brought to AMCA and provide some background on its goals.

56 **Adapting honey-card arbovirus surveillance to US mosquito control programs**

Kristy Burkhalter, ktb3@cdc.gov

Support from the AMCA Research Fund facilitated the collaboration between the Centers for Disease Control and the Placer Mosquito and Vector Control District in Roseville, CA to conduct a field evaluation of honey card-based virus surveillance in the summer of 2018. Furthermore, the outcome of this pilot project spurred interest among other mosquito control districts, leading to additional field trials in 2019. These collaborations were essential to the adaptation of the honey card method from its original implementation in Australia to the surveillance programs typically encountered

in the United States. The optimized method, virus detection results, and operational algorithms that have been developed will be discussed.

57 **Mosquito excreta as a template for genomic epidemiology**

Dana Price, d.price@rutgers.edu, Andrea Egizi, Nicole Wagner, Margherita Porfirio-LaStrapes, Zhuolun Meng, Mehdi Javanmard, Taewon Han

Early detection ensures that vector control efforts are directed to the right location at the right time to avert an epidemic. Currently, the most common monitoring strategies include the reporting of human cases, serological conversion of sentinel hosts, and vector field infection rate assessments. Each of these methods has particular advantages and limitations; surveillance and detection of infected mosquitoes for example is often an early predictor of impending human infection but comes with challenges including the time involved to sort and identify large numbers of mosquitoes while maintaining a cold chain. Given the success of non-destructive means such as saliva collection and preservation to survey mosquito populations, attention soon turned to the feasibility of utilizing not only mosquito saliva but also excreta for virus testing. Via the AMCA Research Fund, we were able to begin a pilot project that utilized shotgun metagenome sequencing to enumerate the breadth of microbes, including pathogenic viruses, in trapped mosquito excreta. Using this foundation, we have further fabricated several devices that employ superhydrophobic surfaces to aggregate and harvest excreta from sugar-fed mosquitoes both in the lab and in the field. Here we will present metagenomic analyses of excreta samples collected using these devices, as well as single-molecule sequencing of culture-dependent template that has allowed us to reconstruct complete bacterial genomes of excreta constituents. We envision work such as this to provide rapid means of pathogen surveillance and functional genomic assessment in the face of a rapidly changing global vector-borne disease landscape.

58 **Infrared Light Sensors Permit Rapid Recording of Wingbeat Frequency and Bioacoustic Species Identification of Mosquitoes**

Dongmin Kim, kimdongmin@ufl.edu, Terry DeBriere, Satish Cherukumalli, Greg White, Nathan Burkett-Cadena

Recognition and classification of mosquitoes is a critical component of vector-borne disease management. Insufficient surveillance data result from labor-intensive/time-consuming tasks, time lags, incorrect classification, and spatial constraints (e.g., remote areas), which undermine surveillance efforts and the corresponding capacity to detect, anticipate, and respond to vector-borne disease transmission. Real-time vector surveillance, based on wingbeat frequency and other parameters, is becoming increasingly important in the development of automated identification systems, but inconsistent data quality and results frequently emerge from different techniques and data processing methods which have not been standardized on wingbeat collection of numerous species. We developed a simple method to detect and record mosquito wingbeat by multi-dimensional optical sensors and collected 21,825 wingbeat files from 29 North American mosquito species. In pairwise comparisons, wingbeat frequency of twenty six species overlapped with at least one other species. No significant differences were observed in wingbeat frequencies between and within individuals of *Culex quinquefasciatus* over time. This work demonstrates the potential utility of quantifying mosquito wingbeat frequency by infrared light sensors as a component of an automated mosquito identification system. Due to species overlap, wingbeat frequency will need to integrate with other parameters to

accurately delineate species in support of efficient mosquito surveillance, an important component of disease intervention.

59 **Application of mid-infrared spectroscopy and chemometrics for the characterization of mosquitoes**

Brian Byrd, bbyrd@wcu.edu, Scott Huffman, Michael Reiskind, Stephanie Richards

Mosquito control interventions are more effective when informed by routine entomologic surveillance. Thus, accurate and rapid species identification remains a critical component of operational mosquito control. Current methods to identify adult mosquitoes rely chiefly on microscopic identification by trained personnel. In some larger mosquito control programs, molecular methods may be used for species and/or pathogen identification and advanced techniques (e.g., age-grading by ovarian dissection) may be used to further assess the mosquito population structure. Each of these methods are labor intensive and subject to a series of operator and/or laboratory errors. Therefore, there is a need for rapid species identification and population characterization (e.g., parity, pathogen prevalence, and insecticide susceptibility) techniques that can be used on a scale that is ecologically, economically, and epidemiologically meaningful. Our current research aims are to develop methods of biochemical discrimination between different mosquito species and physiological states using infrared spectroscopy. Infrared spectroscopy is a sensitive, information rich technique capable of detecting a wide range of molecular signals ranging from subtle changes in protein secondary structure to transmembrane protein-lipid interactions. The resulting spectral data, when coupled with numerical analysis (chemometrics) methods may be used to classify mosquitoes by species or physiologic status. To date, we have successfully applied spectroscopy to identify multiple container-inhabiting *Aedes* (adults and eggs) and *Culex* species (adults) and characterize chronologic and physiologic age. This work and on-going efforts to detect pathogens and further refine analysis parameters will be presented.

Public Outreach in our AMCA Regions Symposium I

60 **Analysis of Mosquito Control Agency Public Education Programs in the United States, 1982 to the Present**

Broox Boze, bboze@vdc.net

In 1979 the American Mosquito Control Association adopted a policy statement indicating that "methods for mosquito control should be chosen after careful consideration of the efficacy, ecological effects, and costs versus benefits of the various options, including public education, legal action, natural and biological control, elimination of breeding sources, and insecticide application." Within a few years, a membership survey was conducted to analyze public education programs implemented by our members and found that 60% of respondents rated public education as "more important" or "equally important" than chemical, biological or physical control." However, survey respondents reported that only 1.7% of their budget was allocated for public education and an average of 30% was allocated for chemical, biological, and physical control.

AMCA's general membership survey, conducted in 2020, indicated that a "lack of public understanding or support of mosquito control" was identified as the number one thing having an impact on our profession in the next three years. "Increasing and improving public outreach" was also listed as our

membership's number one priority. In this presentation I will talk about some of the changes our industry has seen in terms of staff hours committed to public education, percentage of budget earmarked for these purposes, and effectiveness of current education programs.

61 **Communications Overload, The Challenges Of Communicating Mosquito Messaging During A Pandemic**

David Zazra, dzazra@nsmad.com

The recent pandemic generated an overload of public health messages. This created additional difficulties for mosquito control professionals throughout the nation when communicating the potential health risks posed by mosquitoes. Mr. Zazra will discuss some of the challenges experienced by mosquito control entities in the North Central Region, with examples of both successes and failures in cutting through the clutter.

62 **South Pacific public relations snapshot: opportunities and challenges**

Levy Sun, lsun@sgvmosquito.org

There is minimal to no extant literature that discusses the profession of public relations in mosquito control in the United States of America. Since challenges and successes can be regionally focused, one area to examine is the AMCA-designated South Pacific region, which includes Arizona, California, Hawaii, Nevada, American Samoa, and Guam. The researcher conducted a survey of mosquito control agencies and programs in the South Pacific region to determine any patterns and themes that pertain to public relations activities. Two areas of focus are challenges faced by the profession itself in mosquito control, and the messaging around mosquito adulticiding activity, which can be controversial in many communities in the South Pacific region. The findings identify needs for public relations professionals, and any successes and challenges that may provide opportunities for collaboration as a region.

63 **South Atlantic Region: What's working, and what isn't, in our Outreach/Education efforts**

Robin King, rking@cmcd.org

Community outreach and education are core principles for an integrated mosquito management program, but not all mosquito control districts have the same resources to carry out these activities. In this presentation, you'll hear about programs and activities from a variety of districts in the South Atlantic Region. Some of these programs are wildly successful and some are looking for a bit of help from others who have "been there." Regardless of the outreach/education resources at your district, this presentation is offered to provide ideas that may help you

92 **Trying to contact 18 million people from the Rockies to the Great Plains.**

Scott Bradshaw, tooevalleymad@gmail.com

Outreach programs in the West Central Region. Promoting mosquito control and vector control information within Colorado, Kansas, Nebraska, New Mexico, North Dakota, South Dakota, Utah and Wyoming.

Student Competition III

64 **Comparing Aedes mosquito abundance trends using the BG-Sentinel 2, CDC light trap, oviposition cup, and BG-GAT**

Corey Day, coreyallenday96@gmail.com, Rebecca Trout Fryxell

It is critical for mosquito surveillance equipment to provide sensitive estimates of abundance trends so that mosquito control agencies can identify peak periods of mosquito activity. Battery-powered traps are often used for this purpose, in-part because they collect a greater abundance of mosquitoes than passive alternatives like the gravid Aedes trap. However, passive traps may still be useful for measuring mosquito abundance trends, especially if abundance trends observed using passive traps correlate with trends seen in battery-powered traps. Unfortunately, most evaluations of mosquito traps in the literature focus primarily on species composition and the total number of individuals collected, without comparing long-term abundance trends derived from various traps. To address this research gap, we deployed a BG-Sentinel 2, CDC light trap, oviposition cup, and BG-GAT at 10 sites in Knox County, Tennessee for a 20-week period, rotating the traps weekly to avoid placement bias. Along with typical comparisons of species compositions and the overall number of individuals collected, we also compared the seasonal trends in mosquito abundance observed with each trap using mixed effect regression models. The results of this work were used to determine how closely the data observed using inexpensive tools like ovicups and the BG-GAT approximate those observed using standard CO₂-baited traps. The results of this research will be useful for agencies that rely on inexpensive, passive traps to conduct Aedes mosquito surveillance, and for agencies considering the implementation of passive traps in their surveillance programs.

65 **Five novel SNPs in voltage-gated sodium channel gene identified in Aedes aegypti mosquitoes from Florida**

Kyle Kosinski, kyle.kosinski@ufl.edu

Aedes aegypti in Florida is known to be highly resistant to pyrethroid insecticides. We sequenced 107 Ae. aegypti samples collected throughout Florida to examine mutations in a known insecticide resistance gene. We identified five novel single nucleotide polymorphisms (SNPs) and 3 known SNPs. Our sequence data can be used to develop multiplex SNP assay to investigate the SNP frequencies with larger number of samples and illuminate their phenotypic contribution to pyrethroid resistance in Ae. aegypti.

66 **Sterile Insect Technique leads to lower quantity, higher quality, Aedes aegypti adults in Lee County, Florida**

Kate Evans, kgevans@ilstu.edu, Steven Juliano

Our study involved collecting eggs from an SIT treatment area and corresponding buffer and control areas, rearing larvae from the eggs in field-relevant conditions, and comparing the size and number of adult Ae. aegypti that emerged. The number of emerging adults was significantly reduced in the SIT treatment area. Further, while few adult females emerged from the eggs from the SIT treatment area, those that did were significantly larger than females from the control area.

67 **Surveillance of commercial facilities for Culex mosquito production**

Becky Cloud, becky.cloud@emory.edu, Daniel Bartlett, Patrick Irwin

Cryptic habitats are a source of vector mosquito production; however, the importance of cryptic habitat surveillance for *Culex* mosquitoes within commercial facilities has been overlooked. Using a search area determined by historical West Nile Virus (WNV) human case data, we investigated how surveillance of commercial facilities can better quantify sources of vector mosquito production in the Midwest. Our results hope to inform colleagues of the importance of commercial canvassing to identify areas of cryptic habitat.

- 69 **Environmental plastic pollution is giving mosquitoes a new superpower**
Trish Becker, Tbecker4amcd@protonmail.com

Are we unknowingly aiding the serial killers responsible for more human deaths than anything else in history? Female mosquitoes kill almost one million humans worldwide each year. It's known that some mosquitoes lay their eggs in the water contained in plastic. Now, researchers have found that mosquito larvae are eating microplastics. Across the globe, plastics are found in every corner of our lives. I wonder, is our addiction to plastic giving mosquitoes a new superpower?

- 70 **Novel sampling tools and host associations for enzootic vectors of Venezuelan equine encephalitis virus in Florida and Panama**
Kristin Sloyer, ksloyer@ufl.edu, tanise stenn, Mileyka Santos, Eddier Riviera, Jean Paul Carrera, Lawrence Reeves, Anayansi Valderrama, Nathan D Burkett-Cadena

Our goal was to evaluate methods for collecting vectors of zoonotic Alphaviruses and determine their vertebrate hosts in Panama and Florida. Resting shelters effectively sampled *Culex spissipes* and *Culex cedecei*, which fed upon diverse hosts and mammals, respectively. Aspirators effectively collected *Culex panocossa* and *Culex pedroi*, which fed upon diverse hosts and mammals, respectively. Our findings provide tools for efficient sampling of these vectors and insights into their roles in arbovirus transmission.

- 71 **Assessing the Knowledge, Attitudes, and Practices Regarding Chagas Disease Vectors Among National Park Service Employees: A High-Risk Population in the United States**
Antonio Alvarado, aa2757@cornell.edu, Laura Harrington, Emily Mader, Benjamin Burgunder

Southwestern National Park Service (NPS) employees are frequently exposed to triatomines, the vectors of Chagas disease (CD). However, the socio-cultural factors that impact CD transmission in the United States remain unknown. To address this gap, we investigated how NPS employee knowledge and attitudes impacted their triatomine preventive behaviors. We detected high triatomine bite exposure in NPS housing, yet low knowledge of CD. Moreover, increased CD anxiety was associated with increased preventive behavior.

Larval Control & Surveillance I

- 72 **Use of the endangered Gila topminnow (*Poeciliopsis occidentalis*) for larval mosquito control.**
Christopher Reimus, christopher.reimus@pinal.gov

The use of fishes to control mosquito larvae has a long history. However, in some environments, such as the Sonoran Desert in Arizona, the fish most commonly used for mosquito control, the mosquitofish, *Gambusia affinis*, is an invasive species. In an effort to sustainably and responsibly use fish for larval control, the Pinal County Public Health Services District, Vector Control Program explored an innovative option, using a native relative of mosquitofish, the endangered Gila topminnow, *Poeciliopsis occidentalis*, for biological control of mosquito larvae. Doing so required significant effort in partnership with federal and state agencies to obtain appropriate permits to obtain, house, and responsibly stock Gila topminnow. This presentation will outline the ecological consequences of using mosquitofish and describe the challenges of developing a program using a protected species as an alternative for larval mosquito control. The presentation will further discuss the permitting process, fish husbandry, lessons learned, current state, and future goals of the program.

73 **The evolution of Hillsborough County's mosquito fish program – How we adapted to continue our public outreach during COVID-19**

Alexa Patrizio, patrizioa@HillsboroughCounty.org, Mary Geesey

With humble beginnings in 2015, our mosquito fish giveaway program started with a few summer Saturday events piggy-backed off Hillsborough County Pet Resources Events. The mosquito fish giveaways were such a success with our Citizens that the next year Hillsborough County Mosquito Management Services went solo (bye-bye Pet Resources) – all 2016 events were held at County Public Libraries followed by 2017 events held at County Parks. Each successive year saw an increase in Citizen interest resulting in program expansion including a Public Education and Outreach Mobile Lab Trailer in 2020, which debuted at DoDD. The mobile lab, which showcases the mosquito life-cycle and MMS Operations, has proven wildly popular across all generations. In January 2020 we expanded the program to include two, 1000-gallon, hatchery tanks for our mosquito fish in effort to meet the increased demand of our Citizens while maintain a sustainable operation. The arrival of COVID-19 in late-March 2020 changed everything. Keeping our Citizens and employees safe was priority number one while maintaining a vital community education and outreach program. We demonstrated how we were able to adapt to the ever-changing dynamics of COVID-19 through minimizing person-to-person contact by maintaining social distancing, using PPE and devising a drive-thru version of our giveaway event.

Keywords: Public education, public outreach, mosquito fish, mobile lab, COVID-19.

74 **A small-scale field trial of FourStar® Bti CRG for control of *Aedes taeniorhynchus* in the Florida Keys**

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FourStar Bti CRG is a multi-brood controlled release granule for the control of mosquito larvae in a variety of aquatic habitats for up to 40 days or more. The active ingredient is *Bacillus thuringiensis* subspecies *israelensis* (Bti), a naturally occurring soil bacterium that only affects larvae of mosquitoes and similar flies. The Florida Keys Mosquito Control District (FKMCD) currently uses single-use Bti treatments to control *Aedes taeniorhynchus* in field sites throughout the Florida Keys. Since larvicide treatments are required after every rain and tide event, the idea of a long term treatment sounded very advantageous. The purpose of this study was to determine if FourStar Bti CRG could be used as a long term treatment option to help reduce the workload during times of high treatment replication, especially during the rainy season. The study was conducted in the summer of 2021 in field sites known to produce *Ae. taeniorhynchus* larvae in the Florida Keys. There was a total of 20 sites (10

test sites and 10 control sites) where half were woodland pools and the other half were low grassy areas. Pre-treatment larval numbers were collected and then sites were checked twice weekly to monitor product effectiveness. Results will be used to determine if FourStar Bti CRG should be incorporated into the FKMCD operations tool box for *Ae. taeniorhynchus* control.

76 **Detection and management of larval insecticide resistance in catch basin habitats**

Sarah Wheeler, swheeler@fightthebite.net, Marcia Reed, Ryan Wagner

Routine assessment of adult mosquitoes for insecticide resistance is a part of an integrated vector management program. Assessment of larval resistance is equally as important but tends to be less utilized, in part, due to the complexity of performing these assays. Despite the challenges of larval resistance testing, these assays provide critical information for managing a larval mosquito control program. The Sacramento-Yolo Mosquito and Vector District has conducted bench-top cup assays for methoprene and spinosad resistance. Catch basin systems are productive habitats for *Culex pipiens*. Elevated methoprene resistance was observed in one of the District's service areas in 2009, thus the use of methoprene products were discontinued in the area. In 2020 a wide-scale assessment of *Cx. pipiens* resistance levels was conducted to determine whether methoprene use could resume. Bench-top cup assays revealed that methoprene resistance levels had reduced but spinosad resistance had increased. These findings have implications for product selection and rotation. Specific findings and integration of larval resistance testing into a mosquito control program will be discussed.

77 **Evaluation of the In2care® Mosquito Trap against *Culex quinquefasciatus* mosquitoes under semi-field conditions**

Eva Buckner, eva.buckner@ufl.edu, Ana Romero-Weaver, Natalie Kendzierski, Sierra Schluep, Rebecca Zimler, Shawna Bellamy

Culex quinquefasciatus is an important mosquito vector responsible for the transmission of arboviruses like Eastern equine encephalitis virus, St. Louis encephalitis virus, and West Nile virus. Due to insecticide resistance documented in *Cx. quinquefasciatus* populations worldwide, integrated vector management programs may need new strategies to control this species. The In2Care® Mosquito Trap (In2Care Trap), a commercially available trap containing pyriproxyfen (PPF) and *Beauveria bassiana* spores, has been shown to be effective against skip-ovipositing *Aedes aegypti* and *Ae. albopictus*. To see if the In2Care Trap could be used for *Cx. quinquefasciatus* control, we assessed the efficacy of the In2Care Trap in a semi-field setting against locally sourced *Cx. quinquefasciatus*. We found that the In2Care Trap is attractive to gravid *Cx. quinquefasciatus* females, with a significantly higher percentage of egg rafts laid in the In2Care Trap ($34 \pm 6\%$) compared to surrounding flowerpots ($17 \pm 3\%$) ($P = 0.0156$). Adult females successfully autodisseminated PPF to surrounding flowerpots, leading to a significant increase in mosquito emergence inhibition ($13 \pm 2\%$ for negative control vs. $45 \pm 7\%$ for In2Care Trap treatment; $P < 0.001$). Additionally, adult *Cx. quinquefasciatus* exposure to *Beauveria bassiana* spores significantly reduced survivorship ($P < 0.001$). These results suggest that the In2Care Trap may be effective against *Cx. quinquefasciatus* in addition to *Ae. aegypti* and *Ae. albopictus*; however, field evaluations are needed.

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The Effect of Water pH on BTi Toxicity to *Cx. quinquefasciatus*

Timothy McNamara, TMcNamara@agcenter.lsu.edu, Kristen Healy

The Southern House Mosquito (*Culex quinquefasciatus*) is a major arbovirus vector in the Southern United States. A common method of *Cx. quinquefasciatus* management is the use of *Bacillus thuringiensis* var. *israelensis* (BTi) as an aquatic larvicide. The insecticidal activity of BTi is tied to the basic pH of the insect midgut. However, since mosquito larvae develop in a variety of water pHs, the effectiveness of BTi could vary. To determine the effect of water pH on the toxicity of BTi to *Cx. quinquefasciatus*, we conducted a series of trials to determine the effect of pH on BTi induced *Cx. quinquefasciatus* larval mortality. Based on our results, field-realistic variations in water pH can impact larval *Cx. quinquefasciatus* mortality at label rate exposures of BTi.

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A case for considering mosquito control in the entire catch basin network

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Recent studies have failed to detect a reduction in West Nile virus with larger scale catch basin programs. An often untreated source of standing water and mosquitoes that may hinder the effectiveness of catch basin programs is the storm pipe network connected to these structures. Although difficult to inspect, preliminary evidence suggests that storm pipes may hold standing water due to minor shifts in pipe sections, captured debris, and minimum slopes. Observations of mosquito larvae in the pipe network were uncommon but the size of storm pipe networks may make this infrastructure a significant source of mosquitoes.

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Impact of underground storm drain systems on larval ecology of *Culex* and *Aedes* species in urban environments of Southern California

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An extensive network of storm water conveyance systems in urban areas, often referred to as the “underground storm drain system” (USDS), serves as significant production habitats for mosquitoes. Knowledge of whether USDS habitats are suitable for newly introduced dengue vectors *Aedes aegypti* and *Ae. albopictus* will help guide surveillance and control efforts. To determine whether the USDS functions as a suitable larval habitat for *Culex*, *Ae. aegypti* and *Ae. albopictus* in southern California, we examined mosquito habitat utilization and larval survivorship using laboratory microcosm studies. The data show that USDS constituted 4.1% of sampled larval habitats for *Ae. aegypti* and *Ae. albopictus*, and 22.0% for *Cx. quinquefasciatus*. Furthermore, USDS water collected in the summer completely inhibited *Aedes* larval development, but yielded a 15.0% pupation rate for *Cx. quinquefasciatus*. Food supplementation in the microcosms suggests that nutrient deficiency, toxins and other factors in the USDS water led to low success or complete failure of larval development. These results suggest that USDS habitats are currently not major productive larval habitats for *Aedes* mosquitoes in southern California. Our findings prompt inclusion of assessments of pupal productivity in USDS habitats and adult mosquito resting sites in the mosquito surveillance program.

81 **What's the catch? Programmatic comparison of control tools against catch basin breeding**

Angela Caranci, acaranci@northwestmncd.org, Mark Breidenbaugh, Nikia Smith

Due to their ubiquity, variety, and holding capacity, catch basins can quickly become prolific mosquito breeding sources and a resource-intensive focus of mosquito abatement programs. This analysis seeks to consider the comparative efficacy and programmatic sustainability of two novel tools: Sumilarv WSP treatment and In2Care autodissemination trap deployment, against standard IVM practices. Several end point measures are included for discussion to determine comparative 'success.' Observations on the impact of adult mosquito abundance, cost, resident tolerance, and programmatic sustainability are all factors that should be considered when strategizing targeted control efforts.

82 **Long-term solutions to catch basin problems**

Jennifer Henke, JHenke@cvmosquito.org, Gabriela Perezchica-Harvey, Gerald Chuzel

The Coachella Valley Mosquito and Vector Control District has numerous catch basins which have been the primary source of *Culex quinquefasciatus* mosquitoes. Vector control technicians rotate active ingredients to reduce the likelihood of resistance development. Recently, we have examined the efficacy of SumiLarv and MetaLarv XRP applications within our catch basins. Both have been effective at controlling mosquitoes despite the daily influences of irrigation run-off. Here, we will highlight these evaluations and how they have been different from previous work we have conducted for tablets and briquettes.

83 **Comparison of MetaLarv® XRP and Altosid® Ingot for Mosquito Emergence Inhibition in Street Catch Basins in Multnomah County, Oregon**

Peter DeChant, peter.dechant@gmail.com

Street catch basins are important sources of mosquito production in urban and residential areas. Various extended residual formulations are utilized to prevent mosquito emergence from these sources. Extended residual formulations containing S-methoprene inhibit adult mosquito emergence over time based on the juvenile-hormone effect when Methoprene remains present in the water during late-larval instar development. In this study, percent mosquito emergence inhibition (%EI) induced by MetaLarv® XRP and Altosid® Ingot applications to catch basins was measured and compared over the course of thirteen weeks (90 days) in the City of Gresham, Oregon. Each treatment was assigned to one of two adjacent geographic blocks. All accessible catch basins within each block were treated with the assigned material on July 9, 2020. Emergence of pupae collected and isolated from representative basins within each block was measured at three days after treatment and then weekly as conditions allowed. MetaLarv XRP induced an overall average of 98.8% EI (+/- 3.2% SD) and Altosid Ingot induced an average of 97.4% EI (+/- 5.1% SD) over the course of 12 samplings across 90 days. It can be concluded from this evaluation that both products performed similarly, providing high levels of emergence inhibition for at least 90 days.

84 **Season-long efficacy of Sumilarv 0.5G WSP against mosquitoes in urban catch basins**

Caleb Corona, caleb.corona@mgk.com

The impact of vector-borne diseases is felt across the world and can be seen significantly impacting areas where large human populations overlap with the presence of mosquito populations. As

populations have increased over the years, management strategies have been put into place to move water from large precipitation events through city streets to prevent any damage associated with excessive amount of water. While these catch basins provide necessary water management, they do lead to one negative issue. The standing water held in these catch basins provides an ideal oviposition site and serves as a breeding source of Culex and other mosquitoes. Mosquito control professionals have to rely on larvicides to treat problematic catch basins, however, the field applications of larvicides have displayed mixed results due to the complexity of biotic and abiotic factors associated with urban catch basins.

In response to the need of mosquito control in catch basins, MGK has recently provided mosquito control professionals with an option for treating problematic catch basins, by bringing Sumilarv 0.5G & Sumilarv WSP, a product containing pyriproxyfen to the marketplace. One of the main issues facing most larvicides is decreased efficacy of compounds following precipitation events or simply decreased efficacy over the course of a mosquito season. Sumilarv 0.5G & SumilarvWSP has shown the ability to inhibit mosquito emergence from treated catch basins for up to 180 days. Studies using Sumilarv have also shown that flushing of the basins does not impact the efficacy of Sumilarv in the catch basins. This extended efficacy of Sumilarv 0.5G WSP is attributable to the nature of AI and the formulation uniqueness associated with the product.

The overall efficacy of Sumilarv 0.5G WSP paired with the single-use nature of the product makes this product cost-effective to control mosquitoes in catch basins.

The AMCA Research Fund: Coordinating the Efforts of Researchers and the AMCA Community for Greater Impact Symposium II

85 Nontarget impacts of mosquito adulticides on pollinators: Biological factors that affect susceptibility, and indirect effects on parasitism

Dongmin Kim, kimdongmin@ufl.edu, Nathan D Burkett-Cadena, Lawrence Reeves

Application of mosquito adulticides is a beneficial tool for interrupting active arbovirus transmission and in addressing nuisance mosquito issues, and it is a critical element of an Integrated Mosquito Management Program. The goal of such a program is to reduce populations of mosquito pests and vectors while minimizing impacts to nontarget species. Insect pollinators are high profile nontarget organisms that may be affected by mosquito adulticide applications. Considering recent declines in some pollinator populations, and the high public awareness of pollinator issues, it is important to characterize how nontarget pollinators might be impacted, and the factors that affect the susceptibility of pollinators to mosquito adulticides. Much of the work on this topic has focused on the European honeybee, an economically important, albeit nonnative and largely agricultural species. We investigated several factors that may affect the extent to which nontarget native pollinator species (the monarch butterfly and common eastern bumblebee) are exposed or susceptible to mosquito adulticide (malathion) applications. Specifically, we investigated how the size of a monarch butterfly caterpillar affects its ability to feed and develop from malathion ULV spray-exposed milkweed host plants, how the nocturnal roosting habits of eastern bumblebees and monarch butterflies affect susceptibility to malathion ULV spray applications, and how feeding from malathion ULV spray-exposed milkweed

hostplants affects susceptibility to natural enemies (the chalcid egg parasitoid wasp *Trichogramma platneri*) in monarchs.

86 **Transfer of pyriproxyfen to nectar sources and insect pollinators by autodissemination approaches used for mosquito control**

Corey Brelsfoard, corey.brelsfoard@ttu.edu, Sri Jyosthsna Kancharlapalli, Scott Longing

Autodissemination is a method of pesticide self-delivery, which is premised on the use of insects as the delivery agent. This method has recently attracted attention for mosquito control, particularly to target container breeding species such as *Aedes aegypti* and *Aedes albopictus*. The intended goal and appeal of autodissemination approaches are that small amounts of a highly potent IGR are delivered to cryptic mosquito breeding sites. Current field studies suggest that these strategies can be successful at reducing mosquito populations. However, while direct targeting of cryptic locations seems like a significant advantage over large scale applications of insecticides, this could actually be more harmful to non-target organisms by delivering these highly potent long lasting growth inhibitors to the exact places that other beneficial insects visit, such as locations where many anthophilous insects seek nectar sources. Here we discuss experiments that examined for non-specific transfer of pyriproxyfen (PPF) to artificial nectar sources, honey bees, and painted lady butterflies associated with the use of autodissemination approaches. Data suggests male *Ae. albopictus* dusted with PPF deliver lethal doses to artificial nectar sources in laboratory cages and semi-field cages. Furthermore, we demonstrated dusted males indirectly transfer PPF to female *Ae. albopictus*, honey bee foragers, and painted lady butterflies. Transfer of PPF to nectar sources and insect pollinators was also observed with the use of autodissemination stations in semi-field cages. We discuss the results in the context that with any pesticidal approach, there are risks to non-target organisms and the risk may be acceptable in times of mosquito control need, particularly in association with disease outbreaks. However, it's important to understand the level of risk to non-targets and not assume any effects are negligible because PPF is used in small amounts.

87 **Modified attractive toxic sugar baits for *Aedes* vectors**

Jason Pitts, jason_pitts@baylor.edu, Heidi Lindsley, Henry Lyons, Melissa Noreña

Attractive toxic sugar baits (ATSBs) offer an alternative to traditional methods of vector control and have the potential to circumvent insecticide resistance in pest/vector populations and the negative effects in non-target species. Using a sugar-feeding assay, we have identified compounds that shorten the lifespan of adult *Aedes aegypti* and *Ae. albopictus* at low concentrations and are generally recognized as safe (GRAS). In addition, we are using a simple dual-choice paradigm to elucidate odor compounds that are highly attractive to *Ae. aegypti*. We expect that our strategy of utilizing GRAS compounds will lead to the development of modified ATSBs that can be rapidly implemented into existing vector management programs. Our vision is that modified ATSBs will offer a low cost alternative to area-wide insecticide application that can be targeted for use in highly accessed urban areas such as parks and in population-dense neighborhoods, where *Aedes* populations are often among the most numerous and aggressive biting mosquitoes.

88 **Evaluating the Impacts of Mosquito Control on Bumble Bees**

Thomas O'Shea-Wheller, to13870@my.bristol.ac.uk, Timothy McNamara

The impact of mosquito control on non-target organisms is an issue of ongoing concern. Effects upon natural pollinators such as bumble bees are of particular interest, due to their ecological importance, and potential susceptibility. While there is extensive research investigating the effects of agrochemicals upon *Bombus* species, there is a paucity of data relating to the active ingredients used in mosquito control, and their field-realistic exposure levels. As such, our project aims to evaluate the impact of mosquito control adulticides on the model bumble bee species *Bombus impatiens*. Utilizing a combination of individual and microcolony assays, we examine acute LC50 and LD50 values, along with chronic lethal and sub-lethal exposure data for the mosquito control products 'Dibrom®' and 'Duet™'. Additionally, experiments assess field-realistic exposure levels for both products via semi-field deposition experiments. The results from this study aim to provide transferable data for use in risk mitigation protocols, whilst more broadly investigating the potential impacts of mosquito control regimes upon natural pollinators, to further inform best practice.

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The AMCA Research Fund: Now and Into the Future

Edmund Norris, Edmund.Norris@usda.gov, Jennifer Henke

The AMCA Research Fund (AMCARF) has been instrumental in aligning the goals of the AMCA with the broader research community and vice versa. To date, eight research groups have been funded allowing for highly focused and high impact research projects. As we look ahead in the years to come, the AMCAREF would like to feature new directions for the fund as a whole. This presentation will provide an overview of the current state of the AMCAREF and discuss some possibilities for its future growth and development. Audience members should look forward to better understanding the major directions the AMCA Research Fund seeks to follow in the next coming year and brainstorming topics that are important to the AMCA community as a whole. We also aim to survey the attending audience to glean broad interests of the AMCA community and incorporate these into our future Requests for Proposals.

Public Outreach in our AMCA Regions Symposium II

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Twenty-first century mosquito control in Massachusetts- a public debate

Priscilla Matton, brismosqpc@comcast.net

The Commonwealth of Massachusetts has set out on completing a comprehensive study and review to evaluate Massachusetts' mosquito control practices, policies and impacts. One of the biggest concerns identified by those that oppose mosquito control activity is a lack of public outreach and engagement. This talking point has prompted the organized mosquito control districts to review and formalize how and when outreach is conducted. The challenges in reaching our public include compliancy, engagement with those who are not interested, and a varying understanding of the complexity of the issues. There are also misunderstandings between nuisance and vector control, and being proactive versus reactive. Through this work, many have determined that measuring compliance with personal protection recommendations, changing people's positions, or reaching the targeted audience is very difficult. This study has exposed some deep concerns about understanding scientific research and those results.

91 **Public outreach strategies and challenges in the south central region**

Colby Colona, colby@tangimosquito.org, Jennifer Bushnell, Megan McNairn

Mosquito control districts throughout the south central region face both similarities and differences regarding public relations. We have compiled information from organizations across Louisiana and Texas to give a better idea of what tools are used, what challenges are routinely faced, ways this work can benefit mosquito programs, and how Covid-19 has affected outreach.

93 **The experiences of the community mobilization program for the prevention and control of the *Aedes aegypti* mosquito in Puerto Rico**

Julieanne Miranda-Bermúdez, jmiranda@prvectorcontrol.org, Rafael Saavedra-Hernández, Marianyoly Ortiz, Grayson Brown, Lucy Crespo-Valentín

Aedes aegypti is the most serious vector for dengue, Zika, and chikungunya. This container-breeding mosquito is present year-round in Puerto Rico, contributing to the endemicity of these arboviruses. The Puerto Rico Vector Control Unit (PRVCU) aims to increase understanding among citizens of why vectors are a problem, their risk, and responsible breeding site elimination. Since 2017, the PRVCU disseminates information to promote the prevention and control of mosquito *Ae. aegypti* in and around the houses. We focus on *Ae. aegypti* biology and ecology, surveillance and control techniques, arbovirus diseases prevention, among others. To disseminate this information, we use social media, paid and non-paid public campaigns, newsletters, educational videos, a mobile application, education material, house-to-house visits, educational talks and booths, training, loudspeakers, and others. Depending on the audience and project, some outlets and activities are better than others. For example, house-to-house visits performed best for education and dissemination of information on male mosquitoes and *Wolbachia*. In this case, community surveys reached more than 8,000 residents compared to virtual educational talks in which only a few people participated. In our program, we make extensive use of a community engagement formalism known as Knowledge, Attitudes, and Practices (KAP) methodology. In this approach, we assess community knowledge about a given tactic, conduct an engagement effort, and measure the change in attitudes and personal practices that result from our efforts. This has proven valuable in telling us what methods work best and in better focusing our messages. This is one example of our efforts to improve methods for correlating community outreach efforts with the actual impact on vector populations and, ultimately, human disease. In this presentation, we will discuss this topic in more detail and our strategies at improving that methodology.

Unmanned Aerial Systems (UAS) in Mosquito Control Symposium

95 **Larvicide operations with unmanned aircraft systems (UAS) in Placer County**

Scott Schon, scotts@placermosquito.org, Everardo Ortiz, Joel Buettner, Jake Hartle

The Placer Mosquito and Vector Control District (PMVCD) started evaluating UAS larvicide applications back in 2019. Originally, our focus was on liquid applications, and evaluating droplet deposition under different flight parameters. Currently, the District is using a DJI AGRAS MG-1S which is configured for granular larvicide applications. We conducted trials to evaluate application rate and swath for the AGRAS MG-1S spreading system. This data helped provide a good base line to successfully begin applying public health larvicides to mosquito sources throughout the county. This

technology has provided us an effective tool for smaller more precise types of aerial treatments in sources including: irrigated pastures, wetlands, rice fields, and snow melt pools.

96 **Drone based mapping and inspections: Operational savings for habitat treatments**
Peter Brake, pbrake@cmcd.org

Unmanned aerial system (UAS) technology provides a rapid and low-cost solution for mapping both mosquito breeding habitats and aquatic vegetation.

UAS platforms represents a valuable tool for Collier Mosquito Control District's Integrated Mosquito Management program – recent additions of UAS technologies have allowed for increased efficiency through aerial inspections and precision in larvicide applications.

These aircraft come in all sizes and capabilities that capture high-resolution three- dimensional habitat measurements with precision and accuracy. The maps created with drone-acquired imagery are comparable in quality with images from light detection and ranging (LiDAR) surveys with color processing but can be produced at a fraction of the cost and provides the required information in areas of dense vegetation, such as mangrove swamps.

Since its inception in July of 2018, this program has been very successful in producing a vast number of aerial maps that have been easily made and accessible to all individuals due to the ease of operating a UAS and software integration. Mapping with a UAS offers enormous potential to GIS professionals as they provide accurate maps by stitching hundreds of images into three-dimensional imagery software to form ortho-mosaic maps in near real-time to make necessary treatment decisions or collect historical data.

97 **Unmanned Aircraft - Elevating the Management and Mapping of Invasive Species**
Bill Reynolds, breynolds@leateam.com

This presentation will provide participants far reaching uses of Unmanned Aircraft Systems (UAS) and operational applications for mosquito and invasive/noxious weed control. In addition to the aerial applications, aerial imagery used to identify noxious weeds will be presented.

Agencies throughout the United States and worldwide struggle to control non-native plant invasions that disrupt the local ecosystem. Manned aircraft cover large patches, but aerial use is limited by high costs, weather conditions, and overspray. Many studies indicate detecting and controlling small patches provides better control and faster regrowth of preferred plants. Ground applications from vehicles or on foot may target smaller patches, but effectiveness may be limited by accessibility, labor costs, applicator health and safety concerns. Unintentionally, technicians and vehicles also can spread invasives during ground treatments. More than 21 states and 33 agencies are applying herbicide with UAS, commonly referred to as drones. With a payload of 25 pounds and effective, user selectable swath widths between 4-20 feet, the UAS flies at low elevation (10-15 feet) and applies materials with surgical precision. An aerial imagery drone with remote sensors is able to detect emerging invasive weeds and produce classifications of the target weed.

Highlights will include UAS operations by the Hill County Mosquito and Weed District in Montana. The District Supervisor Terry Turner saw a need for the technology, worked to obtain a grant, and implemented the UAS in 2021. His program is utilizing the technology to combat challenging mosquito habitat and map and treat many challenging invasive weeds in the District via UAS, including Russian knapweed (*Acroptilon repens*), Dalmatian toadflax (*Linaria dalmatica*), and Phragmites.

98 **Development of a novel UAS-ready adulticide atomization and delivery system**

Mark Ledebuhr, mark@applicationinsightllc.com

Adulticide applications with UAS have been problematic due to the difficulty of achieving the very low metering volumes required while creating sufficiently fine droplet spectra. Novel metering and atomization methods that do not rely on battery power are being developed and applied to optimize the delivery flow and create an ideal droplet spectrum for dispersal of adulticides in a safe, usable package. The system will be discussed, including current results with active formulations and the path forward to commercialization.

99 **Characterization of the spray distribution of Unmanned Aerial Spray Systems (UASS) and the development of systems for vector control.**

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

The application of vector control products is a crucial component of integrated vector management, a technique we are constantly refining. In pesticide application Unmanned Aerial Spray Systems (UASS) could fill in the gap between manned aircraft and ground equipment. They offer improved efficiency and a more responsible approach from an economical, ecological and human risk perspective. Our team of aerial application experts are developing the necessary protocols and equipment for standardized testing and optimization of UASS in terms of physical characterization.

The available spray systems are under assessment for maximization of on-target effect and minimization of off-target losses. The aim is to identify whether UASS are safe, easy to use, and logistically more effective than standard methods utilizing existing equipment. Currently, no pesticide label has UASS as an approved application technique. For this to happen, the EPA and other registrants will need this spray dispersion and application efficacy information.

The dispersion characteristics will change with aircraft type, application parameters and meteorology. The application parameters and the aircraft type will change depending on the target. For simplicity's sake, we have identified four application scenarios at this stage in the project. Large-scale and small-scale larviciding alongside large-scale and small-scale adulticiding. Data related to the release, descent and deposition characteristics for each system will be presented, alongside the development of an empirical model.

100 **UAS Pilot proficiency safety program**

Marty Scholl, mscholl@fightthebite.net

The Sacramento-Yolo Mosquito and Vector Control District routinely utilizes a variety of UAS models in their fleet that have been selected to accomplish specific tasks. The inclusion of these different UAS models was derived from a desire to capitalize on new technologies to grow the District's UAS program. As the District went through this rapid growth phase it became apparent that while these UAS units were similar, there were enough differences in their operation to cause confusion when pilots

transitioned from one model to another. The differences in the operation of these units highlighted the need to develop a flight proficiency safety program to maintain safe flight protocols to increase safety through pilot awareness. This presentation will detail the development of this program and include the main types and topics of flight control hardware that a flight proficiency safety program needs to address.

- 101 **Drone spraying legal issues**
Jonathan Rupprecht, jon@jrupprechtlaw.com

Spraying from a drone presents tremendous opportunities but there are legal issues to these types of operations that need to be considered before starting. Certain government entities have different opportunities to fly spray drones with less regulation but there are sometimes drawbacks to public aircraft operations. Another major point is how different legal issues play into which aircraft you choose for your operations. Certain drones have more restrictions, inadequate documentation, and longer approval times. It's best to understand these legal issues before purchasing an aircraft.

In this presentation, we will discuss the different legal issues affecting operations of drone sprayers and the different laws that greatly influence your drone purchasing decisions.

Larval Control & Surveillance II

- 102 **Evaluating the impact of the In2Care® Mosquito Trap at low densities**
Estelle Martin, estellemartin@ufl.edu, Decyo McDuffie, Peter Jiang, Daniel Kline

Mosquito-borne viruses are re-emerging and expanding to new geographic locations causing a direct threat to human health. As of today, there are no effective vaccines or therapeutic drugs available for the control of most arboviral diseases, and the prevention and reduction of cases relies mainly on vector control measures. New and affordable tools to control mosquitoes have been developed in the past decade. Yet, for many of these, their efficiency in the field has not been well characterized. In this proposal, we propose to evaluate the efficacy of the In2Care® mosquito trap in reducing the *Aedes aegypti* population in a Gainesville community where it has re-emerged after 26 years of absence. Our goal is to use the In2Care® mosquito trap in conjunction with routine larviciding interventions to reduce and limit the spread of *Aedes aegypti* populations in the Gainesville area as well as determining the lowest trap density at which the In2Care® mosquito trap is efficient in order to reduce the cost of intervention by mosquito control districts.

- 103 **Surveillance of commercial parcel cryptic habitats for *Culex* spp.**
Daniel Bartlett, dbartlett@nwmadil.com, PATRICK IRWIN, Becky Cloud

Cryptic habitats have been investigated, often with focus on residential parcels, to determine areas of peridomestic vector mosquito production. However, operational workflows have determined that commercial facilities, including businesses within residential neighborhoods, harbor cryptic habitats within the Northwest Mosquito Abatement District (NWMAD) in Cook County, Illinois. Many of these sites are directly adjacent to residential parcels and have a constant presence of artificial containers conducive to vector mosquito production. We investigated how refined scouting practices in operations at the NWMAD began to quantify the amount of sites and habitats present. Additionally, we

combined these workflows with spatial analytics and GIS applications to define a targeted search area for an applied research project the following season. This project placed emphasis on sites that store waste tires outdoors with increased focus on source reduction and outreach. By reviewing the results of this multifaceted surveillance we hope to share with colleagues our understanding of these habitats, using GIS to target these efforts and interventions applied by business owners through outreach on the best practices of source reduction.

104 **Key factors for quality control in larvicide bioassay**

Tianyun Su, stevensu1995@gmail.com

Mosquitoes and mosquito-borne diseases remain a public health and well-being burden. Integrated mosquito control by biorational larvicides continues to play crucial role. However, risk of various lavicides based on different active ingredients has been underestimated, evaluated and monitored. Here effort is made to summarize the key factors that impact bioassay outcomes in resistance management, these factors include products used, proper species and life stages, duration of exposure, utensils, larval food, water temperature, result reading, analysis, interpretation and application. The standard operation protocol (SOP) and training can be shared and provided upon request from industries, vector control agencies, and other entities.

105 **Best practices for adding sand to liquid suspension concentrate formulations for UAS applications**

Andrew Rivera, arivera@clarke.com, Mackenzie Harder, Rachel Truttmann

Supplementary vector control can be accomplished via larval source management, with drone application as an efficient and cost-effective mode of treatment. Natular SC, a 22.5% spinosad liquid suspension concentrate, when mixed on sand, could be a single-brood option for drone applications that minimizes drift and increases canopy penetration. Natular SC blended with sand was applied through a drone with an aftermarket hopper to woodland pools with active breeding of Anopheles and Culex mosquitoes. Post-application, the treatment sites were dipped for larvae to determine larval reduction rates. This method proved effective for up to two weeks and may be a potent tool as part of larval source management programs for reducing adult mosquito density.

106 **Working with biocontrol agents against inner city mosquitoes**

Anita Schiller, aschiller@hcp4.net

Harris County Precinct 4's Biological Control Initiative produces multiple mosquito-targeting biological control agents, including predators and parasitoids. Our arsenal includes "mosquito assassins", *Toxorhynchites rutilus*; "Culex destroyer" nematodes, *Romanomermis culicivorax*; predacious cyclopoid copepods; and carnivorous bladderworts, *Utricularia* spp. which are all reared inhouse. They are primarily applied in Harris County parks and conservation preserves where standard mosquito control applications are limited or non-existent and selected to match biology and habitat to best their intended target. Here we share an overview of our 2021 projects at the Houston Arboretum and Nature Center. Its 155 acres function as an inner-city forest oasis complete with walking trails along and over temporarily flooded sunny swamps, shaded natural ponds, and heavily managed, man-made ponds, which all boast their very own mosquito population and diversity. Our efforts there incorporate traditional biological control applications with longevity and establishment studies; static signage and

social media information campaigns generate public interest and serve as educational tools to further interest in natural sciences and STEM disciplines.

107 **Managing mosquitoes in an urban setting – Hillsborough County’s Urban Larviciding Program**

Mary Geesey, GeeseyM@hillsboroughcounty.org, Caitlin Farrell, Karen Ruiz-Ayala

As a result of the increase in widespread resistance of *Aedes aegypti* to mosquito adulticides, we investigated an alternative approach to reduce adult populations in six urban neighborhoods in Hillsborough County, Florida. We mixed Altosid Liquid Larvicide SR-5 with water and applied at a target rate of 3 to 4 oz per acre every two weeks over a sixteen-week period for a total of eight applications per urban neighborhood with an A-1 Mister. To monitor deposition of the larvicide product, we placed bioassay test jars next to ovi position jars, which were deployed the morning before the application of the spray treatment. Each ovi jar contained 250 mL of well water spiked with 3:2 liver/yeast powder mixture and a strip of seed germination paper fastened with a binder clip (collected/replaced weekly). The bioassay test jars weathered overnight (less than 24h) and were collected within two hours of application of spray treatment, which commenced at approximately 3:30 a.m. on treatment nights. The successful deposition of treatment product was confirmed by larval bioassay.

The aim of our urban larvicide program is to demonstrate effective suppression of *Ae. aegypti* populations in six urban neighborhoods.

108 **Resistance, cross resistance and multiple resistance in mosquitoes to biorational larvicides**

Tianyun Su, stevensu1995@gmail.com

Mosquito larvicides derived from microbial organisms and insect growth regulator have been increasingly used to control mosquito larvae. Their relative target specificity, non-target safety and environmentally friendly profile have been well documented. *Bacillus thuringiensis israelensis* (B.t.i.) is a quick and highly target-specific biopesticide against mosquitoes and blackflies, resistance toward intact toxin complex of B.t.i. was rare, however, low to high levels of resistance to individual toxins have occurred in laboratory mosquito populations. The toxins from bacterium *Bacillus sphaericus* is another highly active larvicide against mosquitoes, toward which low to high levels of resistance have occurred in both laboratory and field mosquito populations. The Cyt1A toxin from B.t.i. and Mtx toxin from certain strains of *B. sphaericus* are the key components in resistance management to B.t.i. and *B. sphaericus*. The resistance management strategies have been well developed and implemented. Spinosad derived from *Saccharopolyspora spinosa* has been recently used for mosquito control; high levels of resistance have occurred in laboratory mosquito populations; no management tactics have ever been developed. Methoprene have been used to control mosquitoes for about 40 years, low to high levels of resistance have been occasionally reported in both laboratory and field mosquito populations. Studies on mechanism and management of methoprene resistance are quite meager. Very little attention has been paid to the resistance management in mosquitoes to other insect growth regulators such as pyriproxyfen and diflubenzuron. Additionally co-existing multiple resistance and cross resistance make resistance management more challenging. The prevention of resistance and restoration of susceptibility in mosquitoes to these biorational larvicides are crucial to the success of sustainable integrated mosquito management.

- 109 **Optimizing wide area applications with air blast equipment**
Victoria Hyczyk, vhyrczyk@clarke.com

Wide-area larviciding has emerged as an effective strategy for nuisance and disease control for a wide range of species and habitats, and in particular, larval control of container-breeding species. This session shares observations and operational best practices gleaned from various larval control trials across the United States using air blast equipment and a 22.5% Spinosad-based formulation.

Disease/Vector Studies I

- 114 **Multiple Insecticide Resistance Mechanisms in Urban Populations of *Anopheles coluzzii* (Diptera: Culicidae) from Lagos, South-West Nigeria.**
Ahmed Omotayo, ormorteey32@yahoo.com, Adedayo Oduola

Vector control has proved to be a highly effective component of malaria control, however, widespread development of insecticide resistance in major vectors of malaria has been a major challenge. This study assessed resistance mechanisms in *Anopheles coluzzii* population from Kosofe, Lagos mainland and Ojo Local Government Areas in Lagos, Nigeria where *Anopheles gambiae* s.l is resistant to DDT and Permethrin. WHO susceptibility test was used in determining resistance status of *An. coluzzii* to discriminating doses of DDT and Permethrin. Sub-species of *An. gambiae* s.l were identified using polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (PCR-RFLP) while Allele-Specific Polymerase Chain Reaction (AS-PCR) assay detected *kdr*-West (L1014F) mutation. Biochemical assays were used in determining the activities of metabolic enzymes. DDT resistance was recorded in *An. coluzzii* from the three sites. Mortality rate of mosquitoes exposed confirmed permethrin resistance in Kosofe (50%) and Lagos Mainland (48%) but only suspected in Ojo (96%). All specimens tested were confirmed as *An. coluzzii* with low *kdr* frequency; 11.6%, 16.4% and 6.7% in Kosofe, Lagos mainland and Ojo respectively. Esterase activity was insignificantly overexpressed in Kosofe ($p = 0.849$) and Lagos Mainland ($p = 0.229$) populations. In contrast, GST activity was significantly lower in populations from Lagos mainland (63.650 ± 9.861 ; $p = 0.007$) and Ojo (91.765 ± 4.959 ; $p = 0.042$) than Kisumu susceptible strains (120.250 ± 13.972). Monooxygenase activity was higher in Lagos Mainland (2.371 ± 0.261) and Ojo (1.361 ± 0.067) populations, albeit significantly in Lagos mainland ($p = 0.007$) only. The presence of both target-site mutation and elevated monooxygenase were confirmed in this urban population of *An. coluzzii* from Lagos, Nigeria. Presence of multiple resistance mechanism in *An. coluzzii* call for appropriate resistance management strategy to impact vector control intervention in Lagos.

- 115 **Pyrethroid susceptibility in *Culex quinquefasciatus* Say. (Diptera: Culicidae) populations from Delta State, Niger-Delta region, Nigeria**
Chioma Ojianwuna, ccojianwuna@gmail.com, Ahmed Omotayo, Victor Enwemiwe

The development of insecticide resistance in different species of mosquitoes to pyrethroids is a major challenge for vector-borne diseases transmitted by mosquitoes. Failure of pyrethroids in control of mosquitoes would impact negatively on the gains recorded in control of mosquito-borne diseases in previous years. In anticipation of a country-wide deployment of pyrethroid-

treated nets for control of mosquito-borne diseases in Nigeria, this study assessed susceptibility of *Culex quinquefasciatus* in Owhelogbo, Ejeme and Oria-Abraka communities in Delta State, Niger-Delta, Nigeria to pyrethroids. Three to five days old *Cx. quinquefasciatus* were exposed to Deltamethrin (0.05%), Permethrin (0.75%) and Alphacypermethrin (0.05%) using WHO bioassay method. Polymerase chain reaction (PCR) was employed in characterization of species and *kdr* gene. Results revealed that, *Cx. quinquefasciatus* were generally susceptible (98-100%) to Deltamethrin, Permethrin and Alphacypermethrin in the three communities with the exception of Owhelogbo where resistance is suspected (97%) to Deltamethrin. Knockdown time to Deltamethrin (11.51, 11.23 and 12.68 minutes), Permethrin (28.75, 13.26 and 14.49 minutes) and Alphacypermethrin (15.07, 12.50 and 13.03 minutes) were considerably low for Owhelogbo, Ejeme and Oria-Abraka *Cx. quinquefasciatus* populations respectively. Species identification result showed that all amplified samples were *Cx. quinquefasciatus*, however, no *kdr* allele was found in the three populations. Deployment of pyrethroid-treated nets for control of mosquito-borne diseases in Niger-Delta region of Nigeria is capable of reducing burden of diseases transmitted by *Cx. quinquefasciatus* as well as addressing nuisance value of the vector, however, caution must be entertained so as not to increase selection pressure thereby aiding resistance development.

Keywords: *Culex quinquefasciatus*, Permethrin, Deltamethrin, Alphacypermethrin, Niger-Delta region.

116 **West Nile virus risk estimation and transmission dynamics within a two-vector system in the Chicago suburbs**

Mark Clifton, mclifton@nsmad.com, Haley Johnson, Ana Erkapic, Susan Paskewitz, Lyric Bartholomay

Cook County Illinois and the surrounding suburbs consistently demonstrate some of the highest incidences of human West Nile virus infection in the United States. Mosquito control programs like the North Shore Mosquito Abatement District in Northfield, Illinois routinely trap and test undifferentiated pools of gravid *Culex pipiens* and *Culex restuans* mosquitoes for the calculation of risk indices and to inform when to initiate control measures. The routine combination of *Cx. restuans* and *Cx. pipiens* mosquito pools for arboviral testing, without taxonomic separation, obscures the transmission dynamics and risk estimations of West Nile virus in this two-vector viral system. During the Summer of 2020, *Culex* spp. mosquitoes were taxonomically sorted and tested from both CDC light traps and CDC gravid traps at a high trap density ($n=5$ traps / mile²), high resolution (every other day), low pool size (≤ 10 mosquitoes/pool), and for a period of 74 d during the peak of West Nile virus transmission. This level of detail, duration and fine data resolution has demonstrated that mosquito infection rates are highly variable from day to day with waves of infection building over the season. Interestingly, *Cx. restuans* populations routinely obtain infection rates that average 1.4-fold higher than *Cx. pipiens*, and sometimes exceeding a 4-fold increase. This study underscores the entomological risk posed by gonotrophic state and vector species on a near-daily basis over a season and suggests that *Cx. restuans* are much more of a public health threat than is currently appreciated in the Chicago area.

117 **Rediscovery of *Aedes scapularis* (Diptera: Culicidae) in the Florida Keys**

Lawrence Hribar, lhribar@keysmosquito.org, Carrie Cerminara

Aedes scapularis (Rondani) is one of the most widely distributed mosquitoes in the Americas. Larvae can be found in various temporary and semi-permanent freshwater habitats including swamp margins and crab holes. The first collections of *Ae. scapularis* from the Florida Keys in 70 yr were

made from Boca Chica Key and Key West, Monroe County, Florida, USA. This species is potentially a disease vector. The rediscovery of a mosquito species after so long a time of absence reinforces the need for continuous surveillance and close examination of collected specimens.

125 **Incorporating NASA Satellite Data in Vector Control and Public Health Activities**

Helena Chapman, helena.chapman@nasa.gov, Laura Judd, John Haynes

Over the past decade, the global incidence of vector-borne diseases has increased, leading to significant global morbidity and mortality. Although vector distribution has been influenced by insecticide use, land use changes, and climate change, and access to medical and veterinary care services can hinder clinical diagnosis and management. Multidisciplinary collaborations among scientists and community stakeholders are instrumental for identifying research and practice gaps to apply appropriate vector control interventions that meet local needs. To address this global burden, this work highlights two elements that can improve current vector control practices. First, the One Health concept, which recognizes the interconnectedness among humans, animals, and the environment, represents a framework for a holistic understanding of these intrinsic connections with the surrounding ecosystem. Second, NASA Earth-observing satellite data can serve as a complementary resource to ground-based collections in the assessment of environmental conditions and identification of vector habitats. In this presentation, we will describe the One Health concept and how NASA Earth-observing satellite data can offer in-depth insight about the dynamic processes of the surrounding ecosystem that affect human and animal health. We will share cross-cutting research applications that utilize the One Health concept to enhance understanding of vector-borne disease risks and promote stakeholder engagement to protect community health. Audience members will learn about opportunities to participate in training sessions, contribute to citizen science initiatives, and expand their professional networking within the One Health community.

Doing Data Better: Frontiers in Operational Data Management Symposium

119 **I heart dashboards: How ArcGIS Dashboards have changed the way Collier MCD manages its operational workflow**

Rebecca Heinig, rheinig@cmcd.org, Nate Phillips, Richard Ryan, Keira Lucas

Collier Mosquito Control District has spent the last six years transitioning from paper-based surveillance records to a fully-integrated digital recordkeeping system. Although this solved many problems, it also created new ones, specifically: Now that we had all of this digital mosquito surveillance data pouring into the District in real time, how could we make it quick, easy and even fun for our key decisionmakers to accurately assess current conditions and make timely management decisions? The solution for us was dashboards. In this presentation, we'll show off some of our most useful dashboard interfaces, discuss how they've improved our decision-making processes and share some of the lessons we've learned along the way.

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Are we there yet? : Manatee County Mosquito Control District's experience transitioning from Excel to Super MapVision

Shelley Whitehead, shelly.whitehead@manateemosquito.com

Super MapVision is a data management platform, developed by LeadingEdge, which streamlines the workflow of a mosquito control district to ensure timely exchange of information among departments and to make better informed vector control decisions. However, it is not 'plug and play'; development and testing of this platform to suit an individual district's operational needs demands training, attention to detail, and patience. Historically, Manatee County Mosquito Control District has utilized Microsoft Excel, Google Sheets, MapInfo, and an array of other data management software, which resulted in a cacophony of file types, compatibility problems, and inefficient data pipelines. In 2020, MCMCD began the planning process to combine all operational data into one management platform, Super MapVision. Here, we discuss the development timeline one can anticipate, as well as the unexpected twists that have dotted the journey, from idea to cohesive program.

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Operations-Driven, No-Code App Solutions for Managing Big Data

Ryan Wagner, rwagner@fightthebite.net

Vector control agencies are engaged in the task of managing field inspections while analyzing and maintaining a growing historical database. The unique nature of this endeavor means that agencies looking to streamline operations in the field and in the office may have difficulty finding a ready-made software solution tailored to their needs and will be inclined to resort to outsourcing for custom software development. In an effort to better manage the inspection and treatment of over 200,000 stormwater utilities in its jurisdiction, the Catch Basin Program at the Sacramento-Yolo Mosquito and Vector Control District employed the use of a Google-owned no-code development platform known as AppSheet. As the next step in an evolution from paper maps originally obtained from local municipalities, the custom application was designed to consolidate all the components of the department's tasks previously accomplished by several other methods. The application has shown itself to be a robust, flexible solution capable of handling multiple simultaneous users and the payload of hundreds of thousands of data points. This presentation will discuss the implementation of the AppSheet platform in the context of the District's own transition away from traditional paper maps, and how vector control agencies can avail themselves of no-code resources like AppSheet to expedite operations in the field and data management in the office.

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Playing with Power: automating data-to-decisions with Microsoft PowerQuery

Nicholas DeLisi, ndelisi@stpmad.org, Calvin Smotherman, Kevin Caillouet

Treatment decisions at our district require merging data from multiple disparate sources: trap counts and landing rates from FieldSeeker, virus pooling results from the state, service request counts, weather station reports, and the list goes on. We implemented a series of automated data clean-up and merging processes using PowerQuery within Microsoft Excel, resulting in a combined and condensed hierarchy of zones in need of treatment. Finally, we filter our results through a retreatment interval calculator to determine which zones are eligible to be treated. We have consolidated our day-to-day decisions and can provide consistent service to our residents thanks to PowerQuery.

- 260 **Assessment of the susceptibility status of *Aedes aegypti* populations to pyriproxyfen in a nation-wide monitoring of insecticide resistance**
Kauara Campos, kauara.campos@ufl.edu, Ademir Martins, Cynara Rodovalho, Diogo Bellinato, Luciana Dias, Maria de Lourdes Macoris, Maria Teresa Andrighetti, José Bento Lima, Marcos Obara

To determine the susceptibility profile of *Ae. aegypti* populations to pyriproxyfen, sampling was performed in 132 Brazilian cities, using egg traps. Larvae were exposed to the Diagnostic-Dose and Dose-response bioassays.

Susceptibility alterations were recorded in six populations, with Resistance Ratios from 1.51 to 3.58. The feasibility of conducting a monitoring action on a nation-wide scale was confirmed.

Disease/Vector Studies II

- 124 **An Epidemic Outbreak of West Nile virus in the Shadow of COVID-19 in Tarrant County, Texas 2020**
Nina Dacko, nmdacko@tarrantcounty.com

The World Health Organization declared COVID-19 a global pandemic in March of 2020. It was clear that this was a beast of a disease to deal with. Messaging, resources, and energy from many public health and related departments were then concentrated on prevention, surveillance, and treatment of this devastating illness. As many already knew, just because COVID-19 was the main disease of concern, does not mean other endemic diseases don't flare up as well. In Tarrant County, Texas West Nile virus (WNV) activity had been limited a few years prior to 2020. An epidemic seemed imminent. This presentation will cover clues of an upcoming outbreak, WNV activity during 2020 as compared to the past several seasons, and the challenges of surveillance with limited resources, changing work flows, and proper preparation to keep a vector control program afloat within a public health department during a global pandemic.

- 118 **Quality assurance importance in *Aedes aegypti* rearing**
Johanna Bajonero, johabajonero@gmail.com, Rachel Morreale, Steven Stenhouse, Aaron Lloyd, David Hoel

In order to implement an SIT program, the production of massive quantities of mosquitoes must be assured. Between all the challenges of mass production of male *Aedes aegypti*, maintaining consistent production parameters is essential to produce quality individuals that are able to compete with the wild individuals. For each batch produced in our insectary, we evaluate several parameters to track the fitness of our population. The hatch rate is evaluated with ten samples of 100 eggs per batch produced. To assess the pupa weight, 50 samples of 30 individuals are measured and then individual weight is estimated. Female contamination is evaluated for each sort through pupal identification by checking at least 15 samples of 200 individuals and, if contamination is high, the batch is resorted. Male survival under stress is evaluated for two treatments (irradiated and non-irradiated males), each with 50 individuals and that are fed only with water. Emergence rate is assessed by quantifying whether or not there was successful emergence of 50 individuals and noting mortality. Additionally, we perform periodical evaluations of the sex ratio, the survival of males and females, and

the comparison of the wing length of the colonized males versus the wild type. The current mean values for our rearing conditions are a hatch rate of 81%, individual male pupa weight 2.19 ± 0.21 mg, with female contamination less than 0.03%. The average survival of irradiated males and non-irradiated males do not significantly differ, with an average of 7.5 days to reach 50% mortality under stress conditions. There is a 96.72% rate of successful emergence, 2.24% attempted emergence, and 1.04% that failed to emerge. Our colony has a male bias with a 0.53 sex ratio and our males are just 5% smaller than the wild ones collected in the release area. We have collected information for more than 120 batches in our continuous production system and the information generated have help us to track and solve problems in our production system. While consistent evaluation provides vital information for maintaining a high level of mosquito production, these parameters are also important to consider and monitor for smaller scale rearing as well. Understanding and tracking changes in quality assurance data can provide insight about the overall health of mosquito colonies.

126 **Rift Valley fever virus in humans: A threat to global public health and agriculture**
Seth Britch, seth.britch@usda.gov, Assaf Anyamba, Michael J. Turell, Kenneth Linthicum

Rift Valley fever (RVF) is an acute disease of ungulate livestock and wildlife as well as humans caused by the Rift Valley fever virus (RVFV) which can be transmitted by arthropod vectors such as mosquitoes as well as by direct contact with infected tissues. Humans infected with RVFV can develop extremely high viremias capable of infecting vectors such as mosquitoes. Critically, RVFV has potential for globalization resulting from movement of infected humans into non-endemic regions containing large populations of potentially competent mosquito vectors and abundant susceptible livestock and wildlife hosts which include the US and southern Europe. It is estimated that introduction of RVFV into the US would cost ~\$1billion to the agricultural industry and business. We explore scenarios of escape of RVFV from its endemic range that could be caused by movement of infected humans. We discuss approaches that could be used to mitigate these avenues of spread that include for example surveillance targeted by environmental modeling.

127 **2020 Dengue virus outbreak in Key Largo, FL**
Michael Boehmler, mboehmler@keysmosquito.org

Dengue virus (DENV) is a tropical disease that affects approximately 400 million people per year in over 100 countries. DENV is transmitted from human to human by the bite from infectious *Aedes aegypti* (L.) adults. *Ae. aegypti*, is a domestic pest, ovipositing primarily in close proximity to human domiciles and populated areas. In 2009-2010 Key West, FL suffered an outbreak of DENV that totaled 28 locally acquired cases. Adult population suppression conducted by FKMCD is credited with stopping the spread of DENV. Successful operations and novel techniques utilized during the 2009-2010 outbreak were applied to the most recent outbreak in 2020. The first case of DENV was documented in early March 2020 and was not documented again until mid-June 2020. Using a modified Integrated Pest Management program, we incorporated aerial adulticiding, aerial larviciding, ULV truck mounted sprayers and door to door inspections into one cohesive plan to reduce further spread of DENV. Since 2020, locally acquired DENV cases totaled 67 and no confirmed or suspect cases have been documented in 2021. This presentation intends to elucidate the successes and difficulties the FKMCD disease response methods used to control the spread of DENV and monitor the populations of *Ae aegypti* in Key Largo, FL during the 2020 outbreak of DENV and Covid-19.

- 128 **Effects of pesticide exposure to *Aedes aegypti* and implications on vector competence**
Robert Aldridge, mr.entomology@gmail.com, Kenneth Linthicum, Barry Alto, Roxanne Connelly, Bernard Okech, Blair Siegfried

The impact of pathogen and pesticide on the vector, *Aedes aegypti*, is assessed simultaneously by identifying the LC50 of several larvicides and adulticides, assessing their impact on inferred fecundity through comparison of wing length measurements, then evaluating the difference in rates of dissemination and infection of dengue through the vector. Our results describe important patterns that may influence control measures.

- 132 **The EntoExchange: A mosquito specimen identification and exchange network with data management system**
Dan Killingsworth, dan.killingsworth@gmail.com

The vector management community benefits from sharing surveillance information through trapping data and specimen verification. Collaboration when encountering new or unknown specimens prompts neighboring mosquito control districts and other pest management entities to be on alert for that species and assist with control efforts. Outlets for definitive mosquito specimen identification can be limited or obscure to some operational mosquito management providers. The demand for mosquito specimens in teaching and training collections is extensive and continually in low supply, especially for species outside the practical geographic range of the training facility. As molecular techniques continue to develop, the need for specimen material will be greater than ever. The EntoExchange network supports those needs through volunteer open-source sharing of specimens. It is suggested that each member of the network provide an updatable list of mosquitoes in their area that are obtainable upon request as adults, larvae, and eggs if possible. Seasonal availability for those specimens is included in the data provided by the collector to minimize difficulty in fulfilling requests in a timely manner. Protocols for specific specimen storage and shipping requirements will be provided. Data management for the EntoExchange is supported by EarthSoft EQuIS Alive commercial software which is used worldwide to manage biological, ecological, survey, and taxonomic data. All EntoExchange data will be securely hosted on Microsoft Azure databases, entered through spreadsheets, tablets, or EntoExchange web portals. This is a volunteer network, and no fees or dues are involved. Named logins are required and a level of security, through reference values, is maintained. Get started with an email or web request. Invitations to colleagues to join is encouraged. The EntoExchange will grow over time, as will the diversity of specimens available for research and education.

Enhancing US and Global Mosquito Surveillance with NASA Satellite Data

Symposium

- 133 **Using NASA Satellite Earth Observations to Enhance Public Health Surveillance**
John Haynes, jhaynes@nasa.gov

As the global health workforce recognizes the current and future challenges of emerging environmental threats – such as vector control, water demand and scarcity, and the effects of climate change – the need is understood for novel approaches to complement field epidemiological applications

and advance global discourse. Earth-observing satellite data offer real-time spatial and temporal data about the Earth's ecosystem. When integrated with ground-based observations, they help bridge existing gaps in vector control and public health surveillance. This presentation will provide an overview of the NASA Health and Air Quality Applications program and will highlight selected applied research projects that use Earth-observing satellite data to evaluate human health risks associated with vector-borne and other infectious diseases. This information will demonstrate the value of multidisciplinary collaborations that incorporate satellite- and ground-based data to examine trends on how the Earth's systems are changing due to natural and anthropogenic factors. Session attendees will learn about the program's activities as well as upcoming trainings to strengthen understanding and use of satellite data in environmental health applications.

- 134 **Design and implementation of the Arbovirus Monitoring and Prediction (ArboMAP) system for forecasting West Nile virus across multiple U.S. states**
Michael Wimberly, mcwimberly@ou.edu, Dawn Nekorchuk

West Nile virus (WNV) is the most widespread mosquito-borne disease in the United States, but extreme year-to-year variation in transmission to humans makes prevention and control very challenging. Epidemics of WNV can be triggered by fluctuations in temperature, precipitation, and other climate variables that influence animal vectors, arthropod hosts, and the pathogens they transmit. Identifying and monitoring environmental risk factors can thus support mapping transmission hot spots and forecasting future outbreaks. The National Aeronautics and Space Administration (NASA) produces a variety of environmental monitoring datasets that provide relevant meteorological measurements, but obtaining these data and integrating them into public health surveillance systems can be time-consuming and requires specialized expertise. We developed the Arbovirus Mapping and Prediction (ArboMAP) system to integrate meteorological data from NASA with mosquito and human case surveillance data and generate forecasts of West Nile virus risk. Gridded meteorological data derived from NASA's North American Land Data Assimilation System (NLDAS) are ingested via a cloud-based application implemented in Google Earth Engine. These data are then harmonized with mosquito infection data and human case data collected by state health departments and used to fit models and generate forecasts of human cases during the upcoming WNV season. These forecasts are conditioned on recent meteorological and mosquito infection data and are updated every week as new data become available. Data integration and predictive modeling are implemented in the R environment for statistical computing and are completely automated via a script that produces results and graphics in a formatted report. ArboMAP has been used in South Dakota since 2016, and retrospective validation has demonstrated that the forecasts can capture seasonal variation and predict high versus low transmission years at the beginning of the WNV season. This approach is currently being tested in several other states, including Louisiana, Michigan, and Oklahoma. The results show that it is feasible to integrate routine meteorological data into mosquito-borne disease surveillance, and that incorporating these environmental data improves forecast accuracy compared to predictions based only on mosquito infection data and human case data.

- 135 **Myanmar Malaria Early Warning System: Supporting the global malaria elimination agenda**
Tatiana Loboda, loboda@umd.edu, Dong Chen, Varada Shevade, Allison Baer, Amanda Hoffman-Hall, Maria Tonellato

The wide-spread precipitous decline in malaria burden in Southeast Asia over the past decade has brought forth hopes for successful malaria elimination in this region in accordance with World Health Organization's global malaria eradication agenda. However, in recent years the progress has stalled despite the rising urgency of eliminating artemisinin-resistant malaria from countries in the Greater Mekong Subregion, including Myanmar. One of the challenges is actually presented by the decline in clinical malaria burden: across most of the Greater Mekong countries, malaria is now most frequently distributed in isolated pockets of clinically-silent malaria which requires development of targeted approaches to elimination. These spatially disparate clusters of malaria, primarily distributed in remote parts of Myanmar, are exceedingly difficult to locate given the seasonal variability in malaria outbreaks. The Myanmar Malaria Early Warning System (MMEWS) is designed to support operational and research efforts by medical and public health practitioners to identify likely hotspots of malaria in space and time with a 1 – 2 months forecasting period. Within the MMEWS, we developed a Malaria Burden Potential model which is driven largely by satellite-derived information characterizing environmental conditions as well as population exposure and vulnerability within the theoretical framework of risk assessment. The environmental variability of mosquito habitat suitability – a critical part of the hazard module within the system – is the most dynamic component within the model and is assessed at the 8-day repeat frequency across the country as a whole. Myanmar presents a particularly challenging case study for monitoring malaria-related environmental conditions using Earth Observing systems in the optical and mid-range infrared spectrum due to the dominance of prolonged period of dense cloud cover throughout the monsoon season. We have developed various schemes for gap-filling satellite observations within the monsoon season including vegetation stress and land surface temperature.

136 **Incorporating NASA Earth Science Data into a Health Information System for Supporting Malaria Control**

John Beck, john.beck@uah.edu, Navaneeth Selvaraj, Todd Berendes, Udaysankar Nair, John Painter, Jeffrey Luvall

Malaria is caused by blood parasites transmitted by Anopheles mosquitoes. In malaria-endemic countries, large amounts of public health resources are devoted to controlling mosquitoes, through insecticide-treated bed nets, indoor residual spraying, and eliminating breeding sites. The efficacy of these interventions can be directly influenced by environmental conditions that impact mosquito activity, such as rainfall, temperature, and vegetation. Good quality environmental data are available through countries with satellite-based Earth observations, but the capacity to transfer this data to researchers and public health decision-makers are lacking. Researchers at the University of Alabama in Huntsville (UAH) in collaboration with the U.S. Centers for Disease Control and Prevention (CDC) and NASA have developed a cloud-based web solution to ease the complications of accessing NASA Earth observations from multiple repositories with myriad datasets. A web application called the NASA Earth Observation Import Tool was developed to integrate with public health software already in use by nearly all malaria-endemic countries and provide several key earth observation datasets that are pre-aggregated to regional and sub-regional administrative areas within each country. These services are currently being utilized in support of a web application for the District Health Information Software 2 (DHIS2) system that is used by at least 70 countries. In most of these countries, monthly counts of malaria cases (malaria incidence) are reported to the DHIS2. The frequency and geospatial areas of the earth observation data are designed to match those of malaria cases, thus facilitating comparisons and

correlations of earth observation data with malaria incidence. Because the web application provides DHIS2 with pre-aggregated Earth observation data, the impact on the health information system is minimized, making highly complex data readily available and analyzable. This presentation will discuss the capability of the services, provide examples, and discuss applying the results to intervention efficacy.

- 137 **The Impact of Sustained Malaria Control in the Loreto Region of Peru: A Retrospective, Observational, Spatial Interrupted Time Series Analysis of the PAMAFRO Program**
Mark Janko, mark.janko.collab@gmail.com, Cristina Recalde-Coronel, Andres Lescano, Gabriela Salmon-Mulanovich, Benjamin Zaitchik, William Pan

Background: Although malaria control investments worldwide have resulted in dramatic declines in transmission since 2000, progress has stalled. In the Amazon, malaria resurgence has followed withdrawal of Global Fund support of the Project for Malaria Control in Andean Border Areas (PAMAFRO). A prior evaluation of the PAMAFRO program found it was associated with reduced transmission, but did not attribute reductions to specific interventions in specific places, nor did it account for the effects environmental or climatic variability on transmission, which may be buffered by interventions. We address these limitations by estimating intervention-specific and spatially-explicit effects of the PAMAFRO program on malaria incidence across the Loreto region of Peru, and further consider how interventions influence the effects of the environment.

Methods: We conducted a retrospective, observational, spatial interrupted time series analysis of malaria incidence among people reporting to health posts across Loreto, Peru between January 2000 and December 2011. Model inference is at the smallest administrative unit (district), where the number of diagnosed cases of *Plasmodium vivax* and *Plasmodium falciparum* were determined by microscopy, census data provided population at risk. We include environmental variables associated with malaria transmission, including temperature, precipitation, soil moisture, soil temperature, and windspeed. Environmental data were derived from a hydrometeorological model designed for the Amazon. We used Bayesian spatio-temporal modeling techniques to estimate the impact of the PAMAFRO program, whether the program modified environmental effects on transmission, and further consider the role of climate anomalies on transmission after PAMAFRO withdrawal.

Findings: During the PAMAFRO program, incidence of *P. vivax* declined from 42.8 cases/1,000 people/year to 10.1 cases/1,000 people/year. Incidence for *P. falciparum* declined from 14.3 to 2.5 cases/1,000 people/year over this same period. The effects of PAMAFRO-supported interventions varied both by geography and species of malaria. These reductions were not sustained after the program's end

Operations I

- 138 **Modern Times: Updating mosquito control by creating and improving equipment, tools and methods**
Greg White, greg@slcmad.org, Brad Sorensen, Ary Faraji

Having an assortment of tools and methods aids mosquito control agencies responding to mosquito threats in an ever-evolving world. It is often the case, that new technologies in the realm of mosquito control are adapted from other SLC fields to meet the needs of our industry. One of the goals of Salt Lake City Mosquito Abatement (SLCMAD) is to constantly modernize equipment and tools being

used to find more effective and inexpensive ways of controlling mosquitoes. To accomplish this SLCMAD has worked with collaborators at the Mechanical Engineering College at the University of Utah to design and build new equipment for mosquito control. Staff at the District have also worked to create new tools and update methods to control mosquitoes. This presentation will also discuss how the use of 3D printers and unmanned aerial systems are now a regular part of District operations.

139 **What do the sun, a dog kennel and mosquitoes have in common? The design, implementation and deployment of our BG counter traps**

Mary Geesey, GeeseyM@hillsboroughcounty.org, Leonard Burns

What do the sun, a dog kennel and mosquitoes have in common? Hillsborough County's BG Counter Traps! We will walk through the process of the design, implementation and deployment of our solar powered BG counter traps to our various communities in the County. We will look at the challenges we faced when attempting to secure the traps in the field, which was the highest of priorities. The most desired method to secure a trap is a product that is out of the box. Purchasing a specific dog kennel and the solar panel mounts will provide moderate security of the assets. Because the dog kennels are collapsible, it makes it easy to disassemble, transport and reassemble at a moderate to low cost as compared to custom fabrication. The dog kennel "quick fix" enabled us to deploy traps with little to no delay. The end product has a clean and professional look that houses the stand, motor assembly, battery and a twenty-pound CO2 tank.

Key words: BG counter traps, solar, CO2 tank

140 **Truck trap program: A road less travelled, at Lee County Mosquito Control District**

Milton Sterling, sterling@lcmcd.org

To effectively mitigate nuisance and disease mosquito population, an integrated surveillance program may include the monitoring of adult mosquito flight activities during active periods. There are several mosquito trapping tools that have been developed for monitoring mosquito abundance, but there is one particular method that seem to be overlooked and seldom discussed. The Truck Trap Program. The presentation will focus on the Lee County Mosquito Control District Truck Trap Program operations, successes, and challenges.

141 **Leveraging GIS to Monitor our Active Ingredient Usage**

Rob Chouinard, rchouinard@citrusmosquito.org

Our county mosquito control district is primarily a proactive larviciding based program where we target and kill the mosquitoes in their larval stage of metamorphosis. With our sole aircraft down for the peak mosquito season and record rainfalls in 2021, we had to switch gears and reactively target the adult mosquitoes by using more adulticiding chemicals than we have in recent years. Concerns about exceeding the maximum amount of Active Ingredient (A.I.) per year became real. Leveraging GIS technologies, I developed a procedure to create a heat map from our spray truck logs to show us when we are getting close to reaching our maximum A.I. for a given chemical and date range. 300x300 foot squares cover our whole county. Each square is colored depending on the number of times that square was treated with the given chemical. Transparent for not sprayed or a gradient from green to red, red meaning our max limit. We run the reports on each of the 3 chemicals used once a

week. Using these maps, we can rotate which products are used in which areas and stay under the maximum A.I. per year the EPA label dictates.

142 **Developing and utilizing GIS for mosquito control support and outreach**

Jessica Ber, jessica.ber@FDACS.gov

Geographic Information Systems (GIS) have many uses within mosquito control such as analyzing surveillance data and displaying spray applications. In addition, GIS may also be used for public outreach and mosquito control support. There is a plethora of opportunities to provide valuable information to various groups including the general public. The Division of Agricultural Environmental Services (AES) within the Florida Department of Agriculture and Consumer Services (FDACS) has utilized many applications provided by ESRI (Environmental Systems Research Institute) to expand their outreach ability within FDACS, as well as Florida mosquito control programs and the general public. In 2019, AES designed a new, interactive map which provides the contact information of all state approved mosquito control programs within Florida. This map allows the public to easily search and find their local mosquito control program for assistance. This WebApp is currently available to everyone through the FDACS webpage. AES has also utilized interactive maps and dashboards to increase the efficiency of the Mosquito Control Incident Response Team (MCIRT) during hurricane response. Lastly, several projects are being designed to provide interactive experiences using applications such as ArcGIS StoryMaps and ArcGIS Experience Builder for public education.

143 **Get Dynamic Real-Time Operational Awareness with ArcGIS Dashboards and FieldSeeker**

Chad Minter, chad@frontierprecision.com

FieldSeeker Core is a cloud-based system leveraging the Esri® ArcGIS® Online platform to provide users with simple, intuitive tools for mapping and reporting data related to mosquito control activities. Field and office users benefit from seeing a common up-to-date picture of their operations. Data is centrally located so it may be shared, analyzed, or updated throughout an organization. Dashboards are included with the Web GIS software licensing and present data in an easy-to-read format on a single screen. Dashboards help make decisions, visualize trends, monitor status in real time, and inform their communities. Dashboard examples created by mosquito control districts and public health departments will be shown.

We will also overview recent product updates driven by customer input. Our FieldSeeker Core software (with workflows for Larviciding with storm drain treatments, Surveillance, and Service Request) now has an optional VectorSurv Gateway integration and aerial data import. Adulticiding enhancements include restricted area and proposed treatment area integration with ArcGIS Online and route or area assignments for drivers. We have also gained new experiences in helping districts be more efficient by automating email notification systems or having a dedicated public notification app. Also coming for the next field season is integration with CompassTrac vehicle tracking systems for real-time fleet awareness within FieldSeeker.

- 145 **Collaboration is the Key to Streamlining Technology**
Michael Reynolds, mreynolds@leateam.com

Experience has taught us that the best way to envision future technology is by listening to the needs and challenges of today. Today, many operations are exploring ways to streamline and integrate the ever-growing list of technology solutions supporting their organization. By listening to friends, customers, and other industry stakeholders, MapVision Gen 3 designed efficiencies that support operational progress for individual agencies as well as national data collaboration goals.

This presentation will cover new interfaces to enhance the user experience and the expansion of the Master Management menus to support customizations. By understanding the importance of comprehensive surveillance data to an operation, we'll dive into industry insights and the impact those insights are having on how data flows from agencies. From national systems like VectorSurv to operational field features that monitor and capture ULV truck applications.

- 146 **Replacing the Human Landing Rate with the BG-Counter in the Florida Keys**
Catherine Pruszyński, cpruz@keysmosquito.org

The Florida Keys Mosquito Control District is an independent taxing district with the authority to survey and treat mosquito issues in the inhabited areas of Monroe County, FL. The district has over 266 human landing rate count sites visited daily by one of 16 inspectors. This activity can regularly take up to 2 hours of each inspector's 8 hour shift. The BG-Counter (Biogents AG, Regensburg, Germany) is an autonomous mosquito trap that differentiates mosquitoes from other insects, counts them, and wirelessly transmits the results to a webpage. This technology could enable the district to conduct consistent and standardized surveillance operations while overcoming the requirement of manual inspections. By replacing the human landing rate count station with a BG-Counter, inspectors will have more time available to them to search for larval habitat in other areas. In this experiment, we compared landing rate counts performed by inspectors to the mosquito counts collected by BG Counters.

8th Annual AMCA Arthropod Vector Highlights Symposium

- 173 **Highlights of vector biology**
Sarah Wheeler, swheeler@fightthebite.net

An overview of literature published in 2021, focusing on the field of vector biology.

- 174 **Highlights of vector biology**
Edmund Norris, Edmund.Norris@usda.gov

With the spillover of zoonotic diseases into the human population being particularly in focus due to the pandemic, mosquito control technologies are very topical and remain important for guaranteeing global public health. Unfortunately, resistance to current control technologies and pushback against a variety of insecticides (by both the public and various regulatory bodies) limit the available arsenal of mosquito control professionals and districts. Thankfully, new chemical and non-chemical control strategies are constantly emerging, which will ensure the fight against disease-

spreading arthropods continues. This talk will feature major published works that evaluate some of the emerging tools in the field over the past year. In particular, research articles that evaluate biological, genetic, and chemical control tools will be discussed. Moreover, operational breakthroughs will also be featured that will likely have a significant impact. While far from comprehensive, this overview presentation will highlight the major research studies that are bound to shake up the field within the next decade. Audience members can look forward to learning about updates in research topics such as spatial repellents, new insecticides, and genetically modified mosquito releases, among others!

175 **Highlights of Mosquito Control in Asia**
Peter Obenauer, obenauerp001@yahoo.com

This presentation highlights a selection of papers published on mosquitoes from across the Asian region in 2020 and 2021. Topics included are mosquito biology, control, pathogen transmission and insecticide resistance. Literature databases such as Pubmed, Google Scholar and Agricola were used to find papers from which a selection was made for the presentation. Special emphasis will be placed on Aedes control and the role of Wolbachia in suppression dengue virus transmission.

Social Media and Mosquito Control Symposium

176 **How social media and notifications are promoting transparency about mosquito control efforts**
Robin King, rking@cmcd.org, Andrea McKinney, Keira Lucas, Patrick Linn

Community outreach and education are one of the core principles of an integrated mosquito management program, which ultimately aims to foster public trust. Collier Mosquito Control District (CMCD) uses social media channels to inform the public of potential disease risk and prevention strategies. Additionally, these channels – plus a multi-faceted notification system – provide the “what, when, how, and why” of CMCD’s mosquito control efforts. Details of all treatments are provided using these communication methods to promote transparency and to encourage two-way communication with the public.

177 **Leveraging industry resources for community education**
Miranda Schield, mschield@clarke.com

Industry partners can be a valuable resource for mosquito control programs' social and community education activities. Examples include product-specific support information and other noteworthy communication tools that address common resident questions about integrated mosquito management activities, application methodologies, source reduction activities, vector-borne diseases, environmental impacts, and more. This session will provide real-world examples of the community engagement, social media support, and stewardship tools that are enabling customer community education and engagement efforts.

178 **Mosquito photography for communicating information and science to broad audiences on social media**

Lawrence Reeves, lereeves@ufl.edu

Photography is a convenient and effective means of connecting with broad audiences, from K-12 students to highly educated professionals. The use of striking images can capture the attention of audience members and contribute to the educational value of messages by clearly illustrating the information that is being conveyed. Creating striking and engaging images of mosquitoes can be challenging because mosquitoes themselves are so small, making them difficult to visualize and to photograph. However, there are methods using various equipment, ranging from smartphones to digital SLR cameras and macro lenses that can be used to produce high quality photographs of mosquitoes, designed for engaging audiences on social media. In addition to equipment, identifying the relevant aspects of a message that are likely to resonate with the intended audiences, or to provide them with surprising new information can be beneficial, and using that information to guide the process of creating photographs can be helpful in delivering scientific or mosquito control-related messages. This presentation will cover the elements of photography that are useful to producing impactful images of mosquitoes and how photography can be used to support messaging or the transmission of scientific information through social media platforms.

179 **Creating Social Media Videos that Drive Results: What, Why, and How?**

Mary-Joy Coburn, mjcoburn@glacvcd.org

Social media continues to transform how we communicate. Social media videos are becoming an increasingly important part of brand engagement and information sharing. Gen Z and Millennials, an increasing demographic of decision makers, use videos for information gathering. The unprecedented events of a 2020 global pandemic impacted the amount of video content watched online. If used correctly, social media videos can humanize a public health agency, make vector control more personal, and help staff appear more approachable to the viewer. This presentation will provide tips on video creation using a smart phone, repurposing a video across multiple social media platforms, and highlight examples of videos produced with varying budgets.

180 **SWARM (Strategic Warning & Repetitive Messaging) in Social Media**

Pablo Cabrera, pcabrera@sgvmosquito.org

We live in a world overflowing with messaging from companies fighting for our attention on different platforms. According to Statista advertising expenditure in North America in 2020 amounted to 249.8 billion dollars, with a slight decline due to the COVID-19 pandemic. Despite an initial falloff due to the pandemic, digital advertising spending grew 12.2% in 2020, according to a new report commissioned by the Interactive Advertising Bureau and conducted by PricewaterhouseCoopers (PwC). This only emphasizes the power of digital advertising and the ability to reach your audience where they are.

The San Gabriel Valley Mosquito and Vector Control District is very much aware we are not a multi-million-dollar company, that can spend millions on advertising. One thing we can do is be strategic about the messaging and repeat it so the warning sticks and becomes common knowledge. Advertising by using the appropriate aspect ratio, we create ads to cover as much screen as possible to grab attention. Then

by using targeted lifestyle ads, we can reach the audience we are targeting and get the most bang for our buck.

By focusing on strategy, we keep cost low, expand the campaign to be bite size, and target our most influential audience. This is beneficial in the vector control industry, because messaging can be complicated for the general public to understand and can be overwhelming for small agencies to handle. By repeating the messaging, you allow the audience to process the information and converter them in your advocate.

181 **Importance of plain language**

Jillian Meek, jmeek@pascomosquito.org

Integrated Mosquito Management (IMM) involves, at times, extremely technical protocols, rules, procedures, and applications. How do we as mosquito control staff translate those technical terms to our public? How do we help our citizens understand more about everything we are doing? The answer, plain language. Plain language helps bring your knowledge to a level that is understood by many more outside of our field. The importance of using plain language can provide many benefits to your district. It is imperative to get the buy-in from your citizens, not only so they understand, but to also relieve some of the stresses and difficulties of communicating your efforts. In previous years I have presented on the fun side of social media and messaging, but your plain language posts will be especially helpful when it is time to share higher priority news such as arbovirus transmission, closures, and other announcements. This presentation will elaborate on plain language and give several examples that can be easily applied within your district.

182 **Using Social Media as a Tool for Public and Media Relations**

Michael Mut, mmut@miamidade.gov

The goal of the “Using Social Media as a Tool for Public and Media Relations” presentation is to discuss ways to integrate standard public relations techniques with modern social media practices. The points for discussion will be writing clear, concise press releases, distributing to local, regional, and national media, pitching stories to reporters, and conducting follow-ups. I will provide examples and tips on how to do each, plus illustrate how Miami-Dade County uses social media as a vehicle for p.r. and media relations. The goal is to give attendees a basic understanding of how p.r. works, how to leverage your mosquito control district’s social media channels to enhance what is already being done, and answer any questions about any of the material being presented. Social channels covered will include Facebook, Twitter, and Instagram, with a special emphasis on how reporters tend to prefer Twitter over all the rest. My work in public relations spans more than two decades and has resulted in a vast amount of coverage in print, online, and on TV and radio, for many different industries beyond mosquito control.

Aerial Control/Aviation

183 Angry Birds? (or Not) Impacts and Benefits of Unmanned Aircraft on Avian Populations

Piper Kimball, piper@leateam.com

As more unmanned aircraft systems (UAS) are integrated into mosquito control operations, the impacts, both positive and negative on wildlife, particularly birds, need to be monitored and documented. Historically, one of the most devastating events that can occur during aerial procedures are bird collisions with aircraft. However, even minor disturbances of nesting birds and bird habitat can be considered “take” by the Federal Endangered Species Act, including Incidental Take. Surveillance and treatments for mosquito control work are often conducted within or near habitats that may contain endangered and threatened species. Hence, it is imperative that these activities minimize the negative effects on wildlife populations. Fortunately, unmanned aircraft offer a unique opportunity to complete applications within these locations with minimal environmental disturbances, whether they be temporary (lower noise levels from spray equipment) or long term such as reducing aquatic invasive weeds. Observations from the field will review the importance of documenting bird species and their behavior before, during, and after mosquito control/surveillance efforts. Finally, recommendations for safe piloting procedures when encountering birds will be presented as well as how a proactive approach with protected habitat stakeholders and the public will allow for the continued support of UAS for mosquito control activities in the future.

184 Operational aerial spray trials of Imperium conducted under challenging conditions in two different regions of the US

Mark Latham, manateemcd@aol.com, Kurt Vandock

Imperium is a 2% water-based formulation of deltamethrin utilizing Bayer’s patented FFAST formulation technology. Although similar to DeltaGard, it is specifically labeled for aerial applications against adult mosquitoes. This presentation will discuss two operational applications from Louisiana (high altitude, 300 ft, and large area, 60000 acres) and Delaware (low altitude, 100 ft, and small area, 1200 acres) conducted under challenging conditions of low winds and variable wind direction. Sampling methods utilized in both these trials included bioassay cages containing wild caught local mosquitoes, fluorescent tracer dye added to the tank mix at very low concentrations (0.5% weight/volume) to allow for identification of spray droplets over other airborne pollutants, droplet sampling devices (slide spinners) containing narrow 3 mm slides for improved sampling efficiency, and a temporal sampling method whereby slides at one location are changed out at set intervals in order to ascertain the timing of the passage of different sized fractions of the spray cloud from multiple spray passes over the duration of the spray operation.

185 Utilization of UAS Applications Services in Integrated Mosquito Management

Daniel Markowski, dmarkowski@vdc.net, Broox Boze

Mosquito management programs have long relied upon aerial applications to augment their ground larvicide and adulticide application strategies. However, there has always been a need to target certain sites that were too difficult to access by ground and too large to effectively treat by hand, yet at the same time they were too small for traditional aircraft to efficiently treat. Recently, Unmanned Aerial Systems (UAS) or drones have been identified as an excellent tool to fill that niche. Although

there are many benefits to UAS applications, there are also many pitfalls. Our operations in Utah have experienced their share of these trials and tribulations. In 2021 we have larvicided over 2,000 acres via drone. FAA regulations, swath characterizations, terrain accessibility and routine post-application mapping are many of the early hurdles crossed that will be discussed.

186 **Aerial applications of Bti (Vectobac WDG) for the control of *Aedes aegypti* over a simulated urban environment**

Frances Golden, frances.golden@usda.gov, Benjamin McMillan, Seth Britch, Robert Aldridge, Bianca Moreno, Barbara Bayer, Adam Bowman, Rachel Shepherd, Maurice Solomon, Kenneth Linthicum, Jeffrey Wahl, Mark Breidenbaugh, Karl Haagsma, Jennifer Remmers

In this study we investigated the efficacy of *Bacillus thuringiensis israelensis* (VectoBac WDG) targeting larval *Aedes aegypti* in an urban setting. Due to the tendency of this particular species to breed in cryptic peridomestic habitats, it may be beneficial to spray this larvicide aerially in an attempt to reach these microhabitats. Such microhabitats can include tarps, planters, and toys found in typical urban environments, and may not be easily accessible with a backpack or truck-mounted sprayer. We deployed plastic sentinel cups in a variety of locations in a simulated urban environment located at Camp Blanding in Starke, FL, to determine if the different locations could be reached by 4 trials of aerial sprays with a C-130, with each spray consisting of a single pass over the area. These locations included along roads, inside buildings, in the spray “shadow” of buildings, and in forested areas. We placed control sentinel cups in open areas away from the spray area. Additionally, each location had cups that were deemed “protected” (inside a cardboard box) and “unprotected” (cups sat outside of the cardboard box). After each spray, the larvicide was allowed to settle, after which we capped the sentinel cups and transferred them to the lab at the Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL, where we introduced water, larval food, and 30 *Ae. aegypti* larvae into each cup. For the third and fourth sprays, dye was added to the pesticide to support determination of droplet density in the spray zone. Larval mortality was determined by counting the number of adults that successfully emerged from each cup. The third pass with the C-130 was the only spray of the four to show substantial mortality in sentinels; we discuss larval mortality at the different locations correlated to droplet density and environmental conditions.

187 **From the boneyard to the front lines: How retired military planes have been given a second life in mosquito control**

Edward Foley, Foley@lcmcd.org

The Lee County Mosquito Control District (LCMCD) has recently begun using a new platform of aircraft in its fight against mosquitoes. What started out over a decade ago as part of a comprehensive long-term plan to augment the flight activity of the venerable Douglas C-47, has finally come to fruition for the 2021 mosquito season. In 2015, LCMCD began the process of acquiring two Beechcraft TC-12B from a military aircraft boneyard located in Tucson, Arizona. After a lengthy application process and several trips to the desert, the first of two planes was flown to southwest Florida on January 13, 2017. Over the next four years both planes would receive a comprehensive restoration which brought them back to serviceable condition. To make them suitable for mosquito control, both planes were retrofitted with custom insecticide tanks on the belly of the aircraft. On June 28, 2021, a decade of hard work payed off when N763MC conducted its first successful nighttime

adulticiding mission. In our experience, the TC-12B platform has been a very capable and reliable platform for mosquito control.

189 **Unmanned Aircraft Systems operational use at Lee County Mosquito Control District**
Aaron Lloyd, Lloyd@lcmcd.org

The Lee County Mosquito Control District (LCMCD) is located in southwest Florida. Since its inception in 1958, LCMCD has been a leader in the mosquito control arena by developing new techniques that are effective and 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. Unmanned Aircraft Systems (UAS) are commonly used in mosquito control districts nationwide. There are many uses that have been implemented into an Integrated Mosquito Management program and those uses change with each organization. This presentation will discuss how UAS is currently used in Lee County Florida and future plans for additional technologies that may help identify cryptic mosquito larva habitats.

Product Evaluations & Trials

190 **Advancements toward operational use of the MosID optics and computer vision platform for diverse mosquito identification**
Adam Goodwin, adam@vectech.io, Bala Sai Sudhakar, Sanket Padmanabhan, Jewell Brey, George Constantine, Margaret Glancey, Tristan Ford

Mosquito surveillance, the monitoring of vector abundance, distribution, and diversity, is a critical step to assess mosquito-borne disease risk. Morphological identification and sorting by experts for downstream arboviral testing at mosquito control organizations is essential to an effective entomological workflow. Yet, only a fraction of mosquito control organizations have the resources to dedicate entomologists to spend significant time on day to day identification, leaving many organizations to rely on seasonal staff with minimal taxonomic expertise to guide important downstream intervention strategies. Several technical modalities have been explored for their potential to improve data accuracy and quality throughout the mosquito surveillance workflow. Applied deep learning convolutional neural networks (CNNs) for image recognition have been among the most promising, demonstrating the capability to visually differentiate between mosquito species in controlled optical scenarios and with limited mosquito species datasets. Our team has further developed these algorithms for practical use in MosID, a standardized optical device designed for operational vector surveillance. This includes the algorithmic ability to adjust for image variation observed in assembly or component manufacturing while scaling across several devices, and the ability to determine if a specimen's species is unknown to the system. Current species classification accuracy on data from a single device achieves 98.3+-0.7% over 17 species. When data is transformed to replicate the image variation of ten devices and the CNNs are trained on this augmented data, the standard deviation between the simulated devices is 0.21%. Unknown species detection macro averaged F1-score is 85.6% +- 1.1% across the ten devices, where 21 species that the CNN was not trained on were used for unknown species detection testing. These advancements have enabled the practical deployment of several devices for beta testing in operational vector surveillance this year.

191 **Assessment of two wide-area larvicides for targeting the container-inhabiting mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*.**

Keira Lucas, klucas@cmcd.org, Rebecca Heinig, Rachel Bales, Sara Grant, Nate Phillips, Richard Ryan

With the increasing pressures of resistance to pyrethroid-based adulticides, control of container-inhabiting mosquito species has proved difficult. Wide-area larviciding using the *Bacillus thuringiensis israelensis* (Bti)-based water dispersible granule, Vectobac WDG, has been one method for effectively targeting container-inhabiting mosquitoes in urbanized settings. However, the mixing process and the wear on application equipment caused by water dispersible granules can be cumbersome for the applicator. Recently, the Spinosad-based suspension concentrate, Natular SC, has been made available for urban use to target container-inhabiting mosquitoes. Here, we report the effectiveness and efficiency of the two microbial-based wide-area larvicides, VectoBac WDG and Natular SC, against *Aedes aegypti* and *Culex quinquefasciatus*.

192 **Novel ultra-low weight high-capacity drone release system for sterile mosquitoes**

Hanan Lepek, Hanan@senecio-robotics.com

Sterile mosquitoes are a promising technology for the suppression of local mosquito populations.

Due to short flying distance of the *Aedes albopictus* and *Aedes aegypti*, the target of current sterile insect technique programs, the dispersal of the sterile mosquitoes as close as possible to the local wild population is preferred in order to ensure sterile males are able to find the wild females.

While high density packaging of chilled insects is possible, regulations limit the payload that drones can carry, making it challenging to use conventional cooling systems.

During the presentation a new novel drone release system, representing a breakthrough in combining ultra-low weight chilled payload while able to carry and controllably release more than 100,000 mosquitoes per flight will be discussed. The new technology opens the option to utilize sterile insect technique over large populated areas using low cost, low weight drones making it more accessible and more efficient.

193 **Aerial field cage trials of Imperium® against wild *Aedes taeniorhynchus* and two *Culex* species**

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

Three separate field cage trials were conducted in Lee County between June 2020 and June 2021 to test the efficacy of aerial applications of Imperium® (2% deltamethrin) on wild and naïve populations of *Aedes taeniorhynchus*, and wild populations of *Culex* mosquitoes. The trials were conducted in various wind conditions using three different aircraft (Kingair E90, Airbus H125 and Douglas DC-3). Wild and naïve populations of *Ae. taeniorhynchus* were used in all three tests, and wild populations of *Cx. nigripalpus* and *Cx. quinquefasciatus* were used in different tests. Mortality for the three species and different populations varied between the three tests, reaching 100% under ideal conditions. The results of each cage trial will be presented and the conditions required to produce the highest mortality will be discussed.

194 **Measuring the impact of sunlight on the viability and efficacy of liquid suspension concentrate applications of spinosad**

Rachel Truttmann, rachel.truttmann@gmail.com, Mackenzie Harder, Andrew Rivera

Utilizing air blast sprayers for wide-area larviciding has become an increasingly common form of control for urban container breeding mosquitoes. Fine and very fine droplets are blown up and over homes to deposit into cryptic breeding sources that are not easily reached otherwise. Liquid larvicides like Natular SC, a 22.5% spinosad suspension concentrate, are the preferred formulation type for this kind of larval source management. To determine how long droplets of Natular SC remain viable after depositing onto a surface and drying, a semi-field trial was conducted on common container breeding site materials. The substrates were sprayed with a dilution of Natural SC and water, then left in direct daily sunlight for up to a month. The substrates were then introduced to bioassay cups filled with larvae and the ensuing mortality rates were observed. Potential implications of this study could inform vector control districts on how and when to pre-treat known mosquito breeding sites before an area's wet season begins or mosquito emergence occurs.

195 **An assessment of Valent BioScience's new adulticide formulation for controlling mosquitoes**

Kara Kelley, kkelley@fightthebite.net, Sarah Wheeler, Steve Ramos, Marcia Reed, Samer Elkashef

The development of pesticide resistance can markedly reduce the efficacy of insecticide applications; product rotation is a key strategy in resistance prevention. New active ingredients for the control of adult mosquitoes are rare and often derived from existing agricultural products. To address this lack of novel chemistries Valent BioScience has developed a new formulation designed to fight insecticide resistance. The adulticide combines three different active ingredients including a macrocyclic lactone abamectin, an organic fatty acid, and the class 2 pyrethroid fenpropathrin. This formulation (VBC-60748) is new for mosquito control and currently in the process of EPA registration. The Sacramento-Yolo Mosquito and Vector Control District recently tested VBC-60748 in a side-by-side comparison against DeltaGard (Bayer). The evaluation included three different mosquito populations to determine efficacy of the products against differing levels of insecticide resistance. Two colony populations CQ1 (*Culex quinquefasciatus*; susceptible) and WCP (*Culex pipiens*; resistant), and one field-collected population (*Culex tarsalis*; resistant) were exposed to either VBC-60748 or DeltaGard during two field trials. The results of these evaluations will be discussed within the context of the resistance profiles of these mosquito populations.

196 **The NEVBD Pesticide Resistance Monitoring Program**

Lindsay Baxter, lb694@cornell.edu, Laura Harrington, Elisabeth Martin, James Burtis, Joseph Poggi, Philip Danziger, Emily Mader, Karen Akaratovic, Charles Abadam, Jay Kiser, Penelope Smelser

The Northeast Regional Center for Excellence in Vector-Borne Diseases (NEVBD) is one of 5 CDC-funded centers comprised of researchers, public health experts and vector-borne disease professionals with a shared goal of reducing the risk of vector-borne diseases. Connecting partners from around the Northeast has enabled NEVBD to foster a community of practice that facilitates sharing of knowledge and resources. The pesticide resistance monitoring program was developed in 2019 to augment and expand capacity across the region to gather baseline data and monitor emerging

resistance in medically important mosquito populations. In addition, we evaluate pesticide use and resistance monitoring practices throughout the region through an annual survey. These survey results consistently show that larvicides are the primary method used for vector control in the region and rearing mosquitoes is a common hurdle to conducting in-house resistance monitoring. The NEVBD has developed a specimen submission and testing system, larval bioassays in response to regional needs, onsite training demonstrations, and training materials to assist municipalities in collecting and testing mosquitoes. Results from 2019 through 2021 will be summarized. Briefly, agencies were able to directly submit *Aedes albopictus* and *Culex pipiens* to NEVBD for larvicide and adult bioassays. To-date we have received mosquitoes from 11 of the 13 states in NEVBD's catchment area including larval assay requests from 24 agencies spanning 8 states and adult bottle bioassay requests from 17 agencies spanning 9 states. NEVBD and partnering agencies have identified widespread low-level resistance to methoprene in regional *Culex pipiens* populations. We also detected moderate-high resistance to pyrethroids in several locations and emerging resistance to organophosphates in the mid-Atlantic coastal region.

278 **Automating SIT one larva at a time**
Ariel Livne, ariel@diptera.ai, Victor Levitin, Elly Ordan

Classical, radiation-based SIT has been around for decades. Although SIT is efficient, safe and environmental, rollout has been hindered by operational complications and high costs. Our mission at Diptera.ai is to use technology to make SIT accessible and affordable. The need to rear mosquitoes locally is the main cause of complication and cost for mosquito SIT.

The need for localized production stems from the fragility of adult mosquitoes and the necessity for precise sex sorting. To date, all sex sorting was performed based on external sexual dimorphisms. This is only possible late in development (pupa or adult) as the early developmental stages are asexual.

At Diptera.ai we've developed a sorting platform that utilizes advanced microscopy, machine learning and automation to sex sort wild type mosquito larvae. The ability to separate the males at this early stage not only cuts production costs, but more importantly enables centralized production.

Diptera.ai has an active production site in Israel and is in the midst of its first field implementation treating a couple of Israeli towns.

Lessons from AMCA Best Practices for Integrated Mosquito Management Manual: Protecting the Public from Culex Mosquitoes and Interfering with Arbovirus Transmission Symposium

197 **AMCA's Best Practices for Integrated Mosquito Management manual update**
Jennifer Gordon, jennifer@buglessons.com

In 2018, 94% of domestic arboviral disease cases reported to the Centers for Disease Control and Prevention (CDC) in the United States were caused by West Nile virus (WNV), and the three primary vectors for WNV are *Culex pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis*. Using an integrated approach is the best way to manage mosquitoes and interfere with arbovirus transmission while minimizing the impact on the environment. Integrated mosquito management collects and uses data to

make decisions, utilizes all control methods available, evaluates the efficacy of plans, and frequently engages with the public. Because of the threat posed by Culex mosquitoes, the American Mosquito Control Association received a grant from the CDC to update their Best Practices for Integrated Mosquito Management manual to include information about controlling Culex mosquitoes and interfering with arboviruses transmission. This talk will discuss the importance of integrated mosquito management, highlight information from the updated manual, and discuss how this resource can be used to train new mosquito control personnel and educate a wide-range of stakeholders about best practices for managing mosquitoes.

198 **Overview of Mosquito-borne disease surveillance in Harris County, Texas**

Chris Fredregill, chris.fredregill@phs.hctx.net, Maximea Vigilant

Harris County Public Health Mosquito and Vector Control Division (HCPHMVC) is charged with protecting the residents of Harris County from mosquito-borne diseases. The HCPHMVC monitors mosquito populations primarily for West Nile Virus (WNV) but also tests mosquito populations for Saint Louis Encephalitis (SLE), Dengue (DENV), Chikungunya (CHIKV) and Zika virus (ZIKV).

This Presentation will contain an overview of HCPHMVC current mosquito-borne disease surveillance methodologies. HCPHMVC uses a variety of mosquito trapping and disease surveillance techniques coupled with an in-house Virology laboratory to guide control efforts. Topics to be discussed include current and historical mosquito trap types used, avian surveillance efforts, landing rate counts (in response to declared disasters) and future innovations in collaboration with Microsoft Premonition. Mosquito-borne disease surveillance efforts from the 2021 season will also be discussed.

199 **Ready for action in Salt Lake City: Considerations in setting action thresholds**

Greg White, greg@slcmad.org

Part of a data driven mosquito control program is having a surveillance system in place to monitor mosquito and pathogen activity. Mosquito control agencies then decide when to make applications to control mosquitoes based on the data collected. There are different methods used to create action thresholds for treating mosquitoes. The data used in action thresholds differs from one agency to another based on items like - why the agency was created, what they are trying to accomplish, and what data is available. This presentation will look at how action thresholds have been used in different situations for mosquito and disease control. A detailed examination on how data can be used in setting action thresholds will be discussed using data from the Salt Lake City Mosquito Abatement District, with an emphasis on nuisance mosquito control and reducing West Nile virus transmission.

200 **Borrowing your neighbor's toolkit: Leveraging GIS and remote sensing technologies to track and predict Culex spp. migration**

Kyndall Braumuller, kyndallb@email.sc.edu, Melissa Nolan, Huixuan Li, Sarah Gunter

Culex spp. mosquitoes are the primary vector for West Nile virus and other arboviruses of public health concern. Our recent proof-of-concept pilot study demonstrated the utility of geospatial and remote sensing techniques to monitor Culex spp. abundance and population migration across the distinct ecosystems in a large southeastern metroplex. We incorporated real-world mosquito surveillance, high resolution multispectral imagery classification techniques, vegetation/moisture indices and light detection and ranging (LiDAR) data to develop a block-level predictive model for Culex spp.

abundance. *Culex* mosquitoes were collected at 934 unique trap locations across Harris County, Texas (Houston). Collections occurred from October 2017 to September 2018, comprising a total 10,767 trap nights. Sentinel Hub satellite imagery from four time points were used to generate a supervised classification of multiple land use/land classification types. Finally, machine learning and geospatial statistics were used to generate a highly accurate predictive model for precision-level determination of *Culex* mosquito location and abundance for enhanced vector control management.

This presentation will review the overarching steps to develop our geospatial indices and workflow development. Our reproducible model, based in ArcGIS, a commonly used software for American vector control agencies, serves as a platform for other local vector control agencies to incorporate low-cost geospatial technology to augment their current insecticide application methods. This unique project has brought together a diverse group of experts in an innovative way to tackle an invasive and now endemic public health concern.

201 **Beyond tip it and toss it for *Culex* larvae control**

Patrick Irwin, pirwin@nwmadil.com

Managing *Culex* mosquitoes, like all mosquitoes, is best tackled in the aquatic stage. While most *Culex* management efforts focus on chemical or bacterial larvicides, source reduction/remediation and biological control can be an effective strategy in the right situations. *Culex* mosquitoes comprise several distinct species and are regionally specific; they can utilize numerous habitats for oviposition, so a one size fits all approach is not applicable. Topics discussed will include energy dissipation materials, weed management, rain gardens, small auto repair shops used tire piles and use of larvivorous natural predators. The pro and cons of each remedy will be discussed, from efficacy to the difficulties faced with implementing each strategy.

202 **Current Trends and Future Prospects in Mosquito Larviciding**

Peter DeChant, peter.dechant@gmail.com, Tianyun Su, Bill Reynolds, Ananya Joshi

Integrated Mosquito Management (IMM) is a systematic method of mosquito control which is based on four key elements: surveillance and disease testing, public education initiatives, larval mosquito control and adult mosquito control. Larval mosquito control includes source reduction, the use of natural predators and mosquito larviciding. The keys to successful mosquito larviciding include real-time surveillance-based decision-making, active ingredient selection, formulation selection, and efficient application. Active ingredients and formulation types must be optimized for application to specific habitats and target species. Recent advancements in surveillance through remote (satellite) and up-close unmanned aerial system (UAS) imaging, combined with use of geographic information systems (GIS) have greatly improved data management for larvicide application timing and targeting. Advanced application technologies based on lower rate formulation platforms and application methods have improved efficiency. Very recently, the use of UAS, the WALS® application strategy, underground storm drainage system treatments, and precise calibration of conventional ground and aerial equipment present new opportunities for improved efficiency. The combination of these advancements with application of machine learning and artificial intelligence to the decision matrix offer potential for even more effective larviciding in IMM programs going forward.

203 ***Culex* best management practices for adulticiding and insecticide resistance**

Whitney Qualls, wqualls@amcdf.org

The American Mosquito Control Association received a grant from the CDC to update the Best Practices for Integrated Mosquito Management: A Focused Update to include sections highlighting control of Culex mosquitoes. Part of the process for this review is relying on the support and expertise of a steering committee to provide expert input. This presentation will focus on the updates to the adulticiding and insecticide resistance sections of the Best Management Practices for Culex mosquitoes.

204 **Community Outreach: Planning an Effective Program**
Mary-Joy Coburn, mjcoburn@glacvcd.org

Community Outreach is an integral component of the Best Practices for Integrated Mosquito Management. Alongside surveillance and inspections, vector control districts across the country provide community outreach and education for stakeholders to remove mosquito breeding sources on their properties, and thus reducing mosquito-borne disease transmission in their communities. Most programs may include methods to teach larval habitat reduction and behavior modification like “Tip & Toss.” Education programs may also include the use of personal protection measures, such as repellents and clothing. Though most general guidelines and objectives remain the same, community outreach should be customized to each agency based on the local situation, and budget. This presentation will examine three outreach programs with varying budgets (low, mid-level, and high) from different mosquito control agencies, explore the platforms used to reach their stakeholders, examine the effectiveness of the programs, and discuss successes and challenges faced.

Behavior/Biology I

205 **Silver-doped nanoceria administered via sucrose feeding cause disruption in egg development of Aedes aegypti**
Mona Doshi, mona.mathew@ucf.edu, Craig Neal, Corey Seavey, George Aranjuez, Sudipta Seal, Bradley Willenberg

We have shown that silver-doped nanoceria (AgCNPs) administered via blood feeding halts egg development in Aedes aegypti (Ae. aegypti) and reduces average egg clutch size (doi.org/10.1371/journal.pntd.0008654). In the present study we wanted to determine if nanoceria administered via sucrose feeding would have similar impact on Ae. aegypti egg production. We therefore first fed Ae. aegypti female mosquitoes green-dyed sucrose meals laced with AgCNPs, followed by unadulterated defibrinated bovine blood meal. Only those mosquitoes which had dual colored abdomens (green crop from the sucrose meal and red gut from the blood meal) were selected for oviposition studies. Quantification of eggs production from our current studies suggest that average clutch size is reduced by AgCNP exposure via sucrose feeding. Dilatation studies via confocal fluorescence microscopy on post-oviposition ovaries showed the presence of both secondary and primary follicles in AgCNP exposed mosquitoes that produced less eggs, and AgCNP exposed mosquitoes with zero eggs had either undeveloped primary follicles or arrested primary follicles. Unexposed control mosquitoes showed none of these impacts on egg development. Interestingly, in our related Diptera egg production experiment, female drosophila melanogaster fed AgCNP laced sucrose meals also produced lower number of eggs. Confocal fluorescence microscopy on these fruit fly ovaries revealed that many

ovarioles in AgCNP-fed flies were in earlier stages of development as compared to unexposed controls. Thus nanoceria based agents could offer a new mosquito control tool by interfering with egg production. Our future studies will focus on determining the molecular underpinnings of these nanoceria actions on the development of mosquito eggs.

- 207 **Hunting the hunters: tracking down Western Tree Hole mosquitoes in Salt Lake City**
Neil Vickers, neil@slcmad.org, Kirsten Meredith, Kaia Jay, Kelly Huang, Marcus
Hultmark, Greg White, Ary Faraji

Wind-borne passive scalar cues such as carbon dioxide, body odors and humidity are important in host-seeking behaviors of female mosquitoes and can also play an important role in the location of appropriate oviposition sites by gravid females. Our studies focus on both the field conditions that facilitate scalar-mediated mosquito orientation behavior as well as the identification of novel attractant cues. The western tree hole mosquito (*Aedes sierrensis*) can be found in urban areas of Salt Lake City. Catches of female *Ae. sierrensis* in surveillance traps baited with CO₂ are infrequent but are typically reported by the Salt Lake City Mosquito Abatement District in areas in close proximity to one of the waterways flowing through the city. Wind conditions during periods of mosquito activity in these habitats are characterized by relatively low wind speeds and turbulence. Laboratory two-choice oviposition bioassay studies with gravid *Ae. sierrensis* females revealed a significant preference for laying eggs on substrates in contact with distilled water infused with beetroot peel compared to distilled water alone. Previous studies have shown that *Ae. aegypti* females were similarly attracted to oviposit on such substrates. However, field traps similarly baited with the beetroot peel treatment in these same areas caught few *Aedes* mosquitoes – likely a reflection of the low population numbers in the vicinity due to the exceptionally hot, dry spring and summer of 2021. Future experiments will continue to evaluate oviposition attractants and focus on more extensive sampling along the waterway habitat.

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 (NV) and 2132727 (MH).

- 208 **Contradicting 300 years of excepted mosquito larva respiration theory**
Herbert Nyberg, sales@newmountain.com

For over 300 years it was accepted without contradiction that mosquito larvae respire By exchanging metabolic gases directly with the atmosphere. In analyzing acoustic larvicide, a technique killing mosquito larvae by resonating the gas within their body, we revealed the excepted theory of respiration was incorrect. Our testing and analysis revealed the tracheal system to be isolated and maintain at a pressure above atmospheric. We identified for the first time the structure which isolates the tracheal system from the atmosphere and we also demonstrated there was no obligate need to exchange metabolic guess with the atmosphere. We will be presenting our results has published in Nature's Scientific Reports.

- 209 **Comparison of different sample size cohorts used for the WHO susceptibility Assay in resistance measurements**
Manop Saeung, manop.saeu@ku.ac.th, Theeraphap Chareonviriyaphap

According to the recent WHO test procedures (WHO, 2018), the resistance status of each vector species to insecticides is currently used to evaluate the vector-control intervention. However, ideal number of adult mosquitoes is required to conduct a single set of WHO insecticide susceptibility tests. As it is not always possible to collect enough number of mosquitoes on a single occasion. The objective of this study was to determine whether the three-sample size (5, 10, and 20) cohorts of *Anopheles minimus* can affect bioassay results using WHO susceptible assay. Both field and laboratory test populations of *An. minimus* were tested to find the ideal number of test cohort used for the WHO insecticide susceptibility assay. The assay was conducted with deltamethrin-impregnated papers at discriminating dose (0.05%) and transfluthrin-impregnated papers at lethal dose (0.004%). Each assay was tested for 4 replications with control group. The results showed no different of death among sample sizes against deltamethrin-impregnated papers. Nevertheless, the response of *An. minimus* to transfluthrin-impregnated papers were different depending on sample sizes. However, the result of field population is not yet complete. In conclusion, it might be possible to reduce the sample size to detect and monitoring mosquito resistance for the WHO insecticide susceptibility assay.

210 **The residual effect of Glyphosate (Roundup®) on the development of *Culex quinquefasciatus***

Mahmood Nikbakhtzadeh, mahmood.nikbakhtzadeh@csusb.edu, Yelissa Fuentes

Culex quinquefasciatus is a common mosquito species in southern California and is able to transfer diseases such as West Nile Virus (WNV). California is also a major producer of agricultural products where many lands are devoted to farms and crop products. Glyphosate (Roundup®) is the herbicide which has been frequently used to control broadleaf plants and grasses in southern California. This research investigated the residual effect of Glyphosate on the development of larval stages of *Cx. quinquefasciatus* in laboratory. It has been surveyed whether leaking of low amounts of Glyphosate can impact the development of *Cx. quinquefasciatus* larvae by increasing the daily mortality. This research was also tested the hypothesis that adult mosquitoes that grow in a Glyphosate-infested breeding site might not get the same fitness as the non-affected adults. Measuring the body length and wingspan, fitness of adults emerged from a Glyphosate-infested breeding site was compared with those of the control specimens. Bigger body of an adult is directly related to her higher fat reserves, while longer wingspans gives individuals the capability to fly farther and better seek for their hosts. Those two factors can determine how good the Glyphosate-affected individuals can transfer diseases like WNV to a human population.

212 **Comparison of different sample size cohorts used for the WHO susceptibility Assay in resistance measurements**

Manop Saeung, manop.saeu@ku.th, Theeraphap Chareonviriyaphap

According to the recent WHO test procedures (WHO, 2018), the resistance status of each vector species to insecticides is currently used to evaluate the vector-control intervention. However, ideal number of adult mosquitoes is required to conduct a single set of WHO insecticide susceptibility tests. As it is not always possible to collect enough number of mosquitoes on a single occasion. The objective of this study was to determine whether the three-sample size (5, 10, and 20) cohorts of *Anopheles minimus* can affect bioassay results using WHO susceptible assay. Both field and laboratory test populations of *An. minimus* were tested to find the ideal number of test cohort used for the WHO insecticide susceptibility assay. The assay was conducted with deltamethrin-impregnated

papers at discriminating dose (0.05%) and transfluthrin-impregnated papers at lethal dose (0.004%). Each assay was tested for 4 replications with control group. The results showed no difference of death among sample sizes against deltamethrin-impregnated papers. Nevertheless, the response of *An. minimus* to transfluthrin-impregnated papers were different depending on sample sizes. However, the result of field population is not yet complete. In conclusion, it might be possible to reduce the sample size to detect and monitoring mosquito resistance for the WHO insecticide susceptibility assay.

- 213 **Adding and maintaining colonies with known susceptibility challenges for assessing existing and developing products for resistance management**
Joanna Tyszko, jtyszko@clarke.com

Evaluating mosquito control products against susceptible mosquito populations is an increasingly important practice in the mosquito control industry and academia. As wild mosquito populations continue to demonstrate developing resistance or cross-resistance to common use control products, it has become apparent that novel products with new modes of action should be evaluated against resistant populations in order to assess and validate them as resistance fighters.

This session will share the knowledge and experience of the biology team responsible for obtaining and maintaining two colonies of resistant populations: *Aedes aegypti* PR strain from the CDC, and a pyrethroid-resistant *Culex tarsalis* from Sac-Yolo MVCD. The goal of this session is to highlight best practice and trouble-shooting strategies for rearing and maintaining pyrethroid-resistant colonies over the long-term in a lab setting, as well as providing insight into how these resistant colonies can be used to assess the resistance-fighting potential of potential new mode of action formulations for mosquito control.

Adult Control I

- 214 **The entomological impact of targeted indoor residual spraying (TIRS) against *Aedes aegypti*: results from a cluster-randomized controlled trial in Merida, Mexico.**
Oscar David-Kirstein, odkirstein@emory.edu, Azael Che-Mendoza, Wilbert Bibiano-Marín, Juan Navarrete-Carballo, Anuar Medina-Barreiro, Gabriela González-Olvera, Fabian Correa-Morales, Jorge Palacio-Vargas, Norma Pavia-Ruz, Guadalupe Ayora-Talavera, Pablo Manrique-Saide, Gonzalo Vázquez-Prokopec, ,

There is a desperate need to improve and innovate the chemical control against *Aedes aegypti*, the primary vector of dengue, Zika and chikungunya. A promising approach consists on the selective application of residual insecticides on *Ae. aegypti* indoor resting sites, such as exposed lower sections of walls (< 1.5 m), under furniture, and on dark surfaces (termed targeted indoor residual spraying, TIRS). We quantified the impact of a pirimiphos methyl (Actellic 300CS, Syngenta) TIRS application on *Ae. aegypti* infestation and abundance, as part of a cluster randomized controlled trial quantifying the epidemiological impact of TIRS in reducing arbovirus transmission in the city of Mérida, Mexico. TIRS was applied in ~8,000 houses at a spraying rate of 11 minutes per house. Indoor resting mosquitoes were sampled monthly using Prokopack aspirators on 1,500 houses in control and treatment arms. At baseline (pre-TIRS application), *Ae. aegypti* indices (total adults, females and bloodfed females) were not different between treatment and control arms. Post-spraying, we quantified

a significant reduction between arms of at least 50% in all entomological indices. Significant reductions in entomological indices due to TIRS were also quantified for *Aedes albopictus* and *Culex quinquefasciatus*. Our findings confirm the high and sustained entomological efficacy of TIRS and the value of pirimiphos methyl for the residual control of indoor mosquitoes. This approach could drive a paradigm shift in *Aedes* control by considering *Ae. aegypti* behavior to guide residual insecticide applications and changing deployment to preemptive control (rather than in response to symptomatic cases), two major enhancements to existing practice.

215 Evaluation of a novel macrocyclic lactone-based adulticide to target pyrethroid-resistant mosquito species in Collier County, Florida

Keira Lucas, klucas@cmcd.org, Rebecca Heinig, Nate Phillips, Rachel Bales, Richard Ryan

The Collier Mosquito Control District (CMCD) historically performed mosquito control through aerial application of organophosphate-based adulticides. Today, CMCD follows a robust integrated mosquito management approach using sustainable, evidence-based methods for managing mosquito populations through the variety of tools that exploit the specific habitats and vulnerabilities of the targeted mosquito species. In 2018, CMCD dramatically increased our usage of pyrethroid-based adulticides and, at the same time, identified pyrethroid resistance in two disease vector species, *Aedes aegypti* and *Culex quinquefasciatus*. We also began to see resistance and developing resistance to the organophosphate, naled, in Collier's *Aedes aegypti*. This new knowledge represented a challenge for reducing disease vector populations and to reduce organophosphate-based adulticide usage in the district. For the past 50 years, mosquito control agencies have only had two modes of actions available for the control of adult mosquitoes. Now, the macrocyclic lactone-based adulticide VBC-60748 represents a new mode of action that can effectively target mosquitoes resistant to currently available adulticides. This presentation reports our field-cage trial results using VBC-60748 to target mosquito species resistant to conventional adulticide products in Collier County, Florida.

216 Status of insecticide susceptibility of key malaria vectors in four eco-epidemiological contexts in Cameroon.

Kala Chouakeu Nelly Armanda, nelly_kala25@yahoo.com

Nelly Armanda Kala Chouakeu^{1,2}, Roland Bamou^{1,2}, Edmond Kopya^{2,3}, Marie Paul Audrey Mayi¹, Nchoutpouen Elysée^{2,3}, Parfait Awono-Ambene², Timoléon Tchuinkam¹, Antonio Nkondjio Christophe^{2*}

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Introduction Malaria vector susceptibility to insecticides is a key element in disease transmission elimination strategies. The aim of our study was to evaluate the status of insecticides susceptibility in *Anopheles gambiae* s.l. mosquito vector in four eco-epidemiological settings in Cameroon.

Methods Anopheles larvae were collected from water breeding sites in Kaélé, Tibati, Santchou and Bertoua using the standard dipping method. Larvae were fed until adult stage under insectary conditions and adult females were exposed to insecticides using the standard WHO (2018) test kit for the phenotypic insecticide susceptibility testing. The following insecticides were used: Permethrin, Deltamethrin, Bendiocarb and Malathion. Synergist tests (PBO) were also performed to screen metabolic resistance mechanisms.

Results: A total of 3440 females mosquitoes of the An. gambiae Complex were exposed to Permethrin (n=1322), Deltamethrin (n=1343), Bendiocarb (n=385) and Malathion (n=390) insecticide impregnated papers and to control papers (n=375) in the four localities. At the standard dose (1X), all mosquitoes were resistant to all insecticides except Bendiocarb in Bertoua (95.25%) and Kaélé (100%), and Malathion in Tibati (100%) and Kaélé (100%). Higher doses of Permethrin and Deltamethrin (5X and 10X) significantly increased the susceptibility of mosquitoes in all sites. Results of synergist tests showed a high resistance to Permethrin and Deltamethrin in all sites. Kdr and Ace 1 genes were detected in An. gambiae ss and An. coluzzii in Santchou.

Conclusion: Mosquitoes from Bertoua, Kaélé, and Tibati were found highly susceptible to insecticides in contrary to those from Santchou which were found very resistant. In line with the interpretation of the susceptibility test results, there could be a presence of resistant genes in the malaria vector population of these sites.

Key words: Insecticide susceptibility, malaria vector, Anopheles, eco-epidemiological, Cameroon.

217 **Advances in automated production of sterile male mosquitoes for adult control on large scale**

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The sterile insect technique is gaining evidence as a promising tool for the control and suppression of local mosquito populations by releasing large numbers of sterile male mosquitoes. One of the limiting factors of the technique is the amount of labor required, mainly to manually sex sort and package millions of male only mosquitoes. Typically, mosquitoes are sorted at their pupae level, and require good differentiation in the male and female sizes. In this presentation, our second-generation automated sex separator for high volumes will be discussed. Instead of separating the pupae, they emerge into adults, manipulated on conveying systems, providing flexibility in shipping and rearing. The novel solution, leverage automation and deep learning to provide a simple, affordable and accessible solution for quality control, sorting and packaging of high volumes of male mosquitoes. The presentation will discuss the integration of the solution within a complete mosquito production line, to provide a streamline production process with minimal intervention and disturbances to the fragile insects, targeting a cost-effective solution for city wide scale operations.

218 **Adulticide bioassay - Above and beyond**

Tianyun Su, stevens1995@gmail.com

In combating mosquito nuisance and vector-borne diseases, adulticiding is necessary to cope with urgent situations. Often, adult mosquito populations can bear complicated resistance profile, mainly due to non-public health exposures from agricultural, urban and household use depending on species of interest. Both WHO tube bioassay and CDC bottle bioassay generate mortality data in

response to a fixed dose with fixed exposure time. Conclusion is made for being susceptible, risky to be resistant or being resistant based on certain time-mortality levels. These two assays are great tools to determine existence of resistance in any unknown collections. However, set up and data analysis in both tests can be extended for quantitative and more accurate data analysis and interpretation. If number of test subjects is limited, time-mortality data can be analyzed by probit analysis for dependent data to calculate LT levels, and resistance ratios (RR) can be calculated by comparing with susceptible reference colony. In the situation where sample availability is not an issue, plus the estimate resistance level is high by time-mortality data, LDs are preferred by exposing the test subjects to a dose range with a fixed exposure period that results in approximately 5-95% mortality. Then LDs can be calculated by ptobit analysis for independent data and then RR by comparing with susceptible population. The advantage for calculating RR is to enable quantitative comparison of resistance levels among different populations. Additionally, adulticide bioassay can be further extended to dish assay, surface assay, and wind tunnel assay, etc. and can be done for LT or LD data for RR calculation depending on availability of test subjects and purposes of studies.

219 **Evaluation of Insecticide Resistance in Mosquitoes Collected from Nantucket, MA**
 Broox Boze, bboze@vdc.net, Janet McAllister, Mariah Scott, Daniel Markowski, Julie Darnell

Mosquito control efforts within the Town and County of Nantucket are managed by an advisory committee through the Department of Public Works which oversees contractor services by Vector Disease Control International. Services under this contract include public education, surveillance, disease testing, and larvicide treatment of public properties and private properties as allowed by property owners. No chemical spraying is currently used by the town to control adult mosquitoes, but homeowners are advised that “application of a barrier spray to your yard will provide temporary relief from mosquitoes.”

The objective of this study was to determine the susceptibility/resistance of wild caught mosquitoes to the active ingredients permethrin and deltamethrin which are commonly sold in hardware or garden stores, and utilized by commercial enterprises on the island. In addition, malathion was tested to gather data on an alternative mode of action active ingredient. Bottle bioassays were performed on Culex mosquitoes collected as egg rafts from 5 known larval production sites throughout island. Resistance was found to deltamethrin with only 13% mortality dead at the threshold time and 70% mortality at 2 hours indicating the mechanism behind this resistance is strong. Permethrin and malathion had 96% and 95% mortality at their threshold times and 100%-hundred percent mortality was reached at 60 and 90 min respectively. As public health professionals working within an integrated management framework, establishing baseline data about susceptibility to certain active ingredients is necessary to guide management techniques should the need for wide-area control of adult mosquitoes become necessary.

220 **Sudden increase in mosquito population and West Nile Virus activity in Dallas County from 2019-2021**
 Hadis Hosseinzadehnaseri, hadis@municipalmosquito.com

Dallas-Fort Worth (DFW) is one of the biggest metropolitan areas in Texas, with 254 counties and more than 1,200 incorporated cities. In this review, mosquito population and West Nile

Virus activity in DFW will be examined by looking into data from the most active cities and counties in mosquito control.

The mosquito population increased sharply from 2019 to 2020, Although it's decreasing slightly in 2021. The average number of *Cx. quinquefasciatus* per trap in 2019 was 21.9, which almost doubled to 43.3 in 2020. The average number of mosquitoes thus far in 2021 is 31.7 *Cx. quinquefasciatus* per trap.

The number of positive mosquito pools for Dallas County was 38 in 2019, which jumped to 484 in 2020 (12 times higher than the previous year). In 2021, so far there are 279 positive pools for Dallas County. Although the number of positive pools remained high in 2021, there are fewer reported human cases of WNV. The number of human cases has decreased from 20 in 2020 to 11 in 2021. WNV mortality also decreased from 30% to 9.09% comparing 2020 to 2021.

The reduction in WNV mortality and fewer total human cases in Dallas County in 2021 has been the result of integrated mosquito control and focusing on rapid response to WNV positive pools.

221 **The re-emergence of *Aedes aegypti* in the North-Central Florida**

Peter Jiang, JiangY1@cityofgainesville.org, George Deskins, Sandra Fisher-Grainger

Aedes aegypti was discovered in the City of Gainesville, Alachua county in 2019 after a 26-year absence. This prompted mosquito control programs adjacent to Alachua County to increase their *Ae. aegypti* surveillance. Starting from early 2021, four counties in the North-central area of Florida, including Alachua, Citrus, Hernando and Marion Counties, adopted a standard protocol to conduct *Ae. aegypti* surveillance within each county. The standard protocol includes weekly egg collection (ovitrap), monthly larval collection (door-to-door inspections) and daily adult collection (BG sentinel traps). *Ae. aegypti* was detected in multiple locations of each county, indicating that it is resurfacing in this area where *Ae. albopictus* was the dominate species for the last two decades. *Ae. aegypti* is also showing northward movement through the State. Our results indicate that there is a potential for *Ae. aegypti* to gradually replace *Ae. albopictus* in some of these areas in the future.

222 **Keeping mosquito control relevant: emerging tools of the trade**

Kattie Morris, kmorris@clarke.com, Derek Drews

The science behind mosquito control is always evolving. New tools, concepts and technologies continue to enable operationally effective and highly-scientific mosquito control practices. This session will review some of the emerging tools and concepts being employed for effective research and operational field trials, such as advanced wind tunnel modeling software for optimized aerial performance of a mosquito control formulation; improved tools for understanding the influence of weather conditions of applications; and next-generation or modernized surveillance tools and application equipment. In combination, these emerging technologies and tools are working to deliver the highly sophisticated, data-driven mosquito control programs of the future.

AMCD's Education & Applied Research Benefit Operation Symposium I

- 223 **Overview of Anastasia Mosquito Control Programs**
Rudy Xue, xueamcd@gmail.com

Anastasia Mosquito Control District (AMCD) of St. Johns County is an independent special district and established in 1948. There were 4 substations before 2016. After centralization and moved to a 18 acre complex in the central location of St. Johns county. AMCA has 32 full time employees and 6-8 intern students, and 5-6 seasonal employees with about 5 million budget annually. AMCD programs include customer and professional services, surveillance of arbovirus and mosquito population, operation control included source reduction, larval and adult mosquito control by ground application and aerial application, applied research with several grant funds from Federal, State, and Industries, and education involving employee training and continuing education, public education, and school program. The 5 programs are run by the departments of administration, sciences, ground operation, and aerial application. Due to the excellent programs and employees' efforts and hard work, St. Johns County has been free of any local acquired mosquito-borne diseases for the past 17 years. The district has been recognized as one of leaders in the field of mosquito control and applied research.

- 224 **Anastasia Mosquito Control District's Education and Applied Research Collaborations 2020-2021**
Whitney Qualls, wqualls@amcdf.org

Over the last ten years, Anastasia Mosquito Control District's (AMCD), has increased their Education and Applied Research programs. AMCD conducts in-house employee training, provides an education fund to support the continued education of staff at the University or College level, conducts an annual workshop, and provides facilities to train interns and visiting scientists. Additionally, the Applied Research Program has actively sought out grant funding to promote applied operational research directly benefiting mosquito control. AMCD continues to work with sponsors to develop novel adulticides, larvicides, and repellents for public health mosquito control. This presentation will present an overview on our Education Program and current grants and sponsor related projects conducted by the AMCD Science Team.

- 225 **University of Florida and AMCD Cooperative Research & Education**
Phil Koehler, pgk@ufl.edu, Roberto Pereira, Rui-De Xue

For the past 25 years, the University of Florida and Anastasia Mosquito Control District have had a close history of educating graduate students and conducting applied research on new and novel mosquito control techniques. Results published by students and faculty who have worked on modeling mosquito populations and disease transmission, insecticide efficacy testing, mosquito control insecticides vs honey bees, and attractive toxic sugar baits in cooperation with AMCS will be presented.

- 226 **A collaborative SIT program to control Aedes aegypti in northern Florida**
Kenneth Linthicum, kenneth.linthicum@usda.gov, Robert Aldridge, Jedidiah Kline, Barbara Bayer, Bianca Moreno, Seth Britch, Vindyha Aryaprema, Whitney Qualls, Rui-De Xue, Chen Chao, Daniel Hahn, , ,

Aedes aegypti mosquitoes are a significant public health threat to the world as a vector of Zika, dengue, chikungunya and yellow fever viruses. The control of this domestic mosquito using

classical mosquito control is challenging given the multitude of cryptic immature stage development sites that they can utilize. 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. Given the recent interest in genetically modified mosquitoes and Wolbachia infected mosquitoes for controlling mosquito populations in an SIT approach, a potentially more cost effective and in-house operation involving radiation is being developed between the Anastasia Mosquito Control District, the University of Florida, and the USDA to sterilize locally colonized strains of mosquitoes. We describe the program and present our findings on the feasibility and impact of deploying an SIT program for the control of *Ae. aegypti*.

227 **Development of smart bio-assay cage and evaluation of natural oils as spatial repellents**

Muhammad Farooq, mfarooq@amcdfi.org

For an adulticide application to be effective, spray should be dispersed throughout the control area and pesticide should reach where it is required to control flying mosquitoes. The efficacy of adulticides have traditionally been evaluated with bio-assays cages. In case of lack of mortality of caged mosquitoes, it is hard to decide if the insecticide is not toxic or it was not delivered to the location of the bio-assay cage. Thus, in mosquito adulticide field trials, mortality in bio-assays and spray quantification at the location of the cages are critical parameters to interpret the outcome of the evaluations. Methods have been developed to quantify the amount of adulticide approaching the cages. These methods are very labor intensive. AMCD and University of Florida with funding from CDC, initiated a project to develop a smart cage to capture spray droplets passing through a space, record weather conditions at the time and place of the droplet capture and transmit all data in real time to a remote location. So far, first prototype of a sensor to capture droplets up to a size of 10 micron have been developed and tested with capability to transmit data. The results are a big step in the process of smart cage development.

Due to limited market of spatial repellents, it may not be feasible to get regulatory approvals for new spatial repellents. Thus, a lot of efforts are directed to evaluate natural oils for their potential as spatial repellents. AMCD evaluated Clove oil, Euclyptus oil, Gernoil, Immotelle oil and Lemongrass oil using the True Choice Olfactometer at AMCD repellents laboratory. BG Lure was used as an attractant. The repellent and attractant were released onto one side of the olfactometer and fresh air released to the other side. The volumes of the oils tested were 62.5, 125, 250 and 500 μ L. Overall, the Clove oil, Euclyptus oil, Gernoil, Immotelle oil and Lemongrass oil had 25.3, 16.1, 24.7, 22.1, and 30.5 % less mosquitoes on the repellent side compared to BG lure. Individually, 250 μ L rates of Clove oil, Euclytus oil, and Immortelle oil, 125 μ L of Gernoil and 62.5 μ L of lemongrass oil had best repellency.

Operations II

228 **Managing a sentinel chicken program in the prying eyes of the public**

Jeff Sulzbach, JSulzbach@volusia.org, Miranda Tressler

Managing a sentinel chicken program is essential for an effective arbovirus surveillance program. As with all mosquito control activities, the public sometimes questions our methods and

activities. Managing a sentinel chicken program comes with its own unique set of circumstances. From maintaining a healthy flock, coop design, safety from predators, to food and water needs and basic sanitation. When someone from the public comes across one of our chicken coops, sometimes questions arise.

Volusia County encompasses 1,432 square miles with a population of 553,284. We maintain a flock of 125 chickens, 50 are in the field split between 12 sentinel coops placed throughout the county. The extras are kept in a large coop at our main facility. Blood samples are collected from the field chickens on Monday's and their food and water is refilled. They are check on Tuesdays while picking up traps, and the coops are cleaned, food and water refilled on Friday's. Even with three days of care, we are sometimes questioned about the condition, placement, care or sanitation of our chickens.

Some of our sentinel chicken coops are set on county property that can be accessed by the public. Some are in parks near nature trails, some are behind locked gates and others are on private property. Some of the public's concerns have led to the building of new and larger coops, transfer of coops to other sites, and the installation of signs at each coop describing the program and the reasons behind them. The maintenance of sentinel coops also comes with a monetary cost of time, labor, equipment and food that must be budgeted for.

229 **Novel automated mosquito identification and pooling solution**
Hanan Lepek, Hanan@senecio-robotics.com

Surveillance of local mosquito populations is one of the most important tasks in integrated mosquito management programs. To date, surveillance required experts to perform a vast of non-expert tasks, including manual mosquito separation, identification of common mosquitoes and pooling, resulting in poor optimization of the expert time. These tasks are tedious and simple, yet, experts spend substantial time on performing them. An abatement district must always have an expert on site, and such that lack sufficient man power, are limited in their ability to the extent of not having at all, a surveillance program in their area. In the presentation, a game changer technology for automated pooling and identification will be discussed, showing how artificial intelligence and automation are leveraged in order to optimize the work of experts, performing all the routine and simple tasks by a robotic system, able to continuously separate thousands of mosquitoes, image and identify the common mosquitoes using deep learning, while providing online remote access for experts to identify the more difficult mosquitoes via high resolution images, advancing mosquito surveillance operations to a whole new level.

230 **Brevard County Mosquito Control District: managing mosquitoes on Florida's Space Coast**
Joseph Faella, joseph.faella@brevardfl.gov, Mike Buono, Jonathan Koagel, Jonathan Linder, Shannon Maginnis, Jeff Sabine, Steve Whitt, Keith Minner, Dave Prather, Bill Strittmatter, Julie Black

Brevard County is known to accommodate more than 40 mosquito species and associated diseases along the 72-mile stretch of east central Florida coastline known as the Space Coast. Home of the Kennedy Space Center and multiple state and federal lands, Brevard's diverse habitats include the St. Johns River, Indian River Lagoon, Atlantic coastline, and everything in between. The Brevard County Mosquito Control District employs a fully Integrated Mosquito Management (IMM)

operation, including its infamous mosquito impoundment program, which dates back to the 1950's and currently consists of approximately 28,000 acres of impounded salt marsh within the Indian River Lagoon. With such an extensive wetland management network comes important environmental stewardship obligations as well as associated funding opportunities and partnerships with local, state and federal agencies and universities. Recent and upcoming program enhancements include an 1,800-gallon *Gambusia* hatchery system and stocking program; multiple ground vehicle and watercraft retrofits; unmanned aircraft system (UAS) support; GIS mapping, data management and inventory upgrades; an expanding educational outreach program; and a new aircraft hangar/ biology laboratory facility.

- 231 **A summary analysis of BG Counter traps for mosquito population monitoring in Illinois**
Michelle Selander, mselander@clarke.com, Lauren Lavezzi

What happens when a large, regional surveillance network containing more than 300 adult mosquito traps introduces next-generation BG Counter technologies? This session will provide a real-world, operational comparison of surveillance data gathered through traditional New Jersey Light traps and next-generation BG Counters in Illinois, and offer best practices and recommendations for reliable data collection and informed control decisions.

- 232 **An evaluation of flight distance for the salt marsh mosquito species *Aedes taeniorhynchus* and *Aedes sollicitans* in Volusia County**
Rebecca Owers, ROwers@volusia.org, Miranda Tressler, Jeff Sulzbach, Daniel Gomez, Dean-Peter Claassen, Nicolas Lee, Caroline Efstathion

Volusia County has more than 50,000 acres of salt marsh habitat and is home to the nuisance species, *Aedes taeniorhynchus* and *Aedes sollicitans*. These species are the primary pests of Volusia County Mosquito Control with a majority of resources focused on the surveillance, larvicide, and adulticide operations. Additional local information is needed to more efficiently target operations and treatments for these species that can travel long distances into residential areas.

To accomplish this, a self-marking device adapted from Saddler et al. 2019 was created which can be placed at larval sites in the salt marsh. Once the mosquitoes emerge from the water, they move through this device and are marked with fluorescent pigment. Before this adapted device was placed in the field it was tested in a controlled screen room with trays of larval mosquitoes to confirm efficacy. The results showed that the device successfully marked emerging mosquitoes in a controlled setting. The first attempt was 62% successfully marked and after further adaptations the screen room trials resulted in 100% successfully marked adult mosquitoes.

The field evaluation will have the device set in an area that historically has seen high abundance of these species. A series of ABC and BG traps will be set at 1mi, 5mi, 10mi and 20mi intervals to catch the marked mosquitoes which can be seen using a UV light under the microscope. It should be noted that all environmental conditions will be recorded as these will influence the field results. From these results a further evaluation will determine if traps need to be set at greater distances. The field evaluation will be repeated using different fluorescent pigment in order to establish a time frame for the mosquito to travel.

- 233 **From dry ice to CO2 bottles: changing trap methods at Tangipahoa MAD**
Colby Colona, colby@tangimosquito.org

For years, dry ice has been used as the bait in CDC traps set at Tangipahoa Mosquito Control District (Hammond, Louisiana). However, dry ice in our region is not only very expensive, but is difficult to obtain. This can cause disruptions in surveillance operations and can lead to delays. A technique applied from Salt Lake City MAD uses CO2 bottles instead of a cooler with dry ice. We tested this technique last season and found that it is more cost effective and easier to maintain. We will continue to use this method in the future.

- 234 **COVID-19 and the Vector-Borne Disease Prevention Workforce**
Courtney Youngbar, cyoungbar@astho.org, Paris Harper-Hardy

State and territorial health agencies are an important line of defense against both endemic and emergent vector-borne disease (VBD) threats, often responsible for prevention as well as coordination of responses at local and broader levels. In winter 2020-2021, the Association of State and Territorial Health Officials (ASTHO), launched a survey to the members of the National Association of Vector-borne Disease Control Officials (NAVCO), regarding their current capacity for surveillance, prevention, education, and planning, and how the COVID-19 pandemic has affected those capacities. Data collection was conducted via an online survey containing multiple choice and open-text questions. NAVCO members were contacted directly via email to participate in the survey. The survey was completed in March 2021. Drawing on results from a 2007 ASTHO survey to NAVCO members, and a 2017 survey to state/territorial environmental health directors and vector control program managers, the data shows that the COVID-19 struck vector-borne disease programs at their most vulnerable points as participants indicate that their heaviest impacts were due to loss of staff time and reduced capacity to implement vector surveillance and/or control activities. Both 2007 and 2017 participants indicated high levels of need for more staff positions in several areas, especially public health entomology, and other challenges representing longstanding areas that can be addressed to support health agencies in their efforts to address changing patterns in VBDs. The findings of this new survey highlight opportunities to promote the implementation of VBD programs through strategic planning and strengthening the social connectedness of the vector-borne disease prevention workforce.

- 235 **Alternative power sources for operating CDC light traps for mosquito surveillance**
Jim Cilek, james.e.cilek.civ@mail.mil

Several portable, rechargeable Li-Ion cell phone power banks were compared with standard 6v gel lead acid batteries as alternative power sources for operating mosquito surveillance equipment. In laboratory trials, ToughTested® (TT)16000 and 24000, Goal Zero Venture™ 70, and Griffin Survivor® units either met or exceeded that of 6v batteries when operating CDC suction light traps (with incandescent light on) for an average of 24 h. No significant difference was found when traps were continually operated when powered by either the TT16000 or Goal Zero Venture 70 units compared with 6v batteries (at ~57 h). The TT24000 unit was the only Li-Ion power bank that exceeded this threshold at an average of ~73 h. In field studies, there was no significant difference in species diversity or abundance of mosquitoes between the above four power sources when operating CDC light traps for 24 h compared with 6v batteries. Our results indicate that portable Li-Ion cell phone power banks ≥10050 mAh may be suitable replacements for 6v gel lead acid batteries when operating these light

traps, especially if weight and size constraints influence the capacity and scope of mosquito surveillance.

The views expressed in this presentation are those of the author and do not reflect the official policy or position of the Navy and Marine Corps Public Health Center, Navy Bureau of Medicine and Surgery, Department of Defense, or the US Government. The author is an employee of the U.S. Government. This work was prepared as part of his official duties. Title 17, U.S.C., §105 provides that copyright protection under this title is not available for any work of the U.S. Government. Title 17, U.S.C., §101 defines a U.S. Government work as a work prepared by a military Service member or employee of the U.S. Government as part of that person's official duties.

236 Impacts of the sterile insect technique (SIT) on populations of *Aedes aegypti* (L.) on Captiva Island, FL

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

In 2017, Lee County Mosquito Control District began the foundational step of collecting data to establish an entomological baseline in preparation for sterile insect technique (SIT) applied to a local population *Aedes aegypti* on Captiva Island, FL. Ongoing and consistent population monitoring in both the planned release area on Captiva Island and the control area on Sanibel Island provided insight on the population dynamics, seasonal influences, and locality of *Ae. aegypti*. Additionally, several mark-release-recapture studies were informative to understand the dispersal, longevity, and survival of released sterile male *Ae. aegypti*. After the establishment of mass rearing, irradiation, and quality assurance parameters, operational sterile male releases began in June 2020 following suppression of wild populations in the field. Through twice weekly releases of sterile males that have increased as the program has scaled up, we have been able to show suppression of wild *Ae. aegypti* in the release area. Overall, in the area where releases have been occurring, adult surveillance through BG Sentinel trapping has shown a drastically reduced population of adults which is complemented by a major reduction in eggs collected through oviposition cup monitoring. Conversely, in the control area where sterile male releases are absent, wild populations of *Ae. aegypti* have followed the expected activity patterns established through the entomological baseline collections and have reached the highest activity levels since surveillance began in 2017. Likewise, the oviposition data have shown that the control area experienced similar egg collections to previous years. The use of SIT at LCMCD has been extremely promising so far. As mass mosquito production continues to increase, LCMCD is increasing the release area and is expanding efforts to suppress the population of this invasive mosquito at the pilot site.

AMCD's Education & Applied Research Benefit Operation Symposium II

237 Establishing the use of action thresholds by mosquito control programs and characterization of surveillance parameters

Vindyha Aryaprema, varyaprema@amcdfl.org, Madeline Steck, Steve Peper, Muhammad Farooq, Rui-De Xue, Whitney Qualls

Effective mosquito control relies on timely and spatially effective interventions. Hence, understanding and establishing evidence-based action thresholds to initiate interventions are becoming critical in integrated mosquito management systems. The use of action thresholds will help manage the development of insecticide resistance as well as program logistics by reducing the number of unwanted intervention cycles. Identification of key surveillance parameters and the use of historical surveillance data are imperative to establish reliable action thresholds. An initial survey was conducted to, (i) identify mosquito control programs which have set action thresholds, (ii) characterize surveillance parameters. A questionnaire inquiring about program demographics, surveillance (mosquito, environmental, disease) and mosquito control efforts was developed on the Qualtrics online platform and distributed on-line. A total of 224 responses were received from mosquito control programs in 18 countries which belong to five continental regions; North America (n=152), Australia (n=11), Asia - 2 countries (n=15), Africa -13 countries (n=39), Europe - 1 country (n=1) and 6 US military units (n=6). We identified 68% (North America), 60% (Australia), 78% (Asia), 35% (Africa) and 50% (US military units) of the responding programs which perform mosquito control have set mosquito control action thresholds. The use of surveillance parameters which are important in establishing action thresholds was characterized and the findings will be discussed during the presentation.

238 **Arboviral surveillance through mosquito pools and sentinel chickens at AMCD**
Steve Peper, speper@amcdf.org

Arboviral surveillance is an important aspect to any mosquito control or public health operation, especially with the recent rise in vector-borne diseases in the United States. Testing of mosquito pools and the use of sentinel chickens are two common practices utilized for arboviral surveillance. In 2020, the Anastasia Mosquito Control District (AMCD) established an in-house mosquito arbovirus and sentinel chicken testing program. AMCD tested over 540 mosquito pools for West Nile virus (WNV), eastern equine encephalitis virus (EEEV), and St. Louis encephalitis virus (SLEV) in 2020 – no pools testing positive – and as of August 2021 have tested over 530 mosquito pools with one pool testing positive for WNV. AMCD also screened 54 sentinel chickens each week from June through December in 2020 for WNV, and 60 sentinel chickens starting in April of 2021 with screening ongoing. AMCD compares in-house results from sentinel chicken testing with results provided from the Florida Department of Health state lab’s testing of the same samples. In 2020, AMCD tested over 1400 sentinel samples and detected one positive bird for WNV. Comparative results varied from the state lab. Thus far, no sentinel chicken samples have tested positive in 2021 for WNV. In-house testing provides results at a much faster rate (same day verses about 3-5 days from the state lab). However, the downside to in-house testing is being limited to only screening for WNV while the state lab is able to screen for exposure to more arboviruses.

239 **Factors affecting the vector competence of mosquitoes for arboviruses**
Michael J. Turell, mturell@erols.com

There are more than 100,000,000 cases of disease caused by mosquito-borne viruses every year. Some of the more important of these include diseases caused by infection with dengue, chikungunya, yellow fever, West Nile, eastern equine encephalitis, Japanese encephalitis, or Rift Valley fever viruses. For most of these viruses, only a relatively small number of mosquito species are involved in the transmission process. In order to develop more efficient programs to reduce, or hopefully to eliminate, these diseases, we need to have a better understanding of the factors that affect vector

competence, or the ability of mosquitoes to become infected with and to transmit these arboviruses. Not only do different mosquitoes differ in their ability to transmit a particular virus, but different populations of the same species can differ greatly in their ability to transmit a particular strain of virus. Similarly, different strains of the same virus can differ significantly as to which mosquito species can serve as a vector. Environmental temperature can not only affect which mosquito species are present but can greatly affect the vector competence of those species. Although different animals may produce similar viremias, the species of animal may significantly affect the ultimate infection rate in a species ingesting that level of viremia. Finally, the presence of other organisms, such as microfilaria or Wolbachia can significantly increase or decrease the ability of a mosquito to transmit a virus. A better understanding of how each of these factors affect vector competence will allow us to more efficiently control mosquitoes, and more importantly, reduce the incidence of disease.

240 **Going 'Autocidal' in Anastasia**

Stephen Dobson, sdobson@mosquitomate.com

This presentation will highlight recent research performed in collaboration with the Anastasia Mosquito Control District (AMCD). The summary will include recent advances, related to autocidal mosquito control technologies, including experimental data, community engagement, regulatory decisions and communication between industry, abatement district managers and federal/state decision makers. Autocidal methods employ 'self-delivering' strategies of 'mosquitoes against themselves.' The presentation will include the Wolbachia approach, which is based on repeated, inundative releases of Wolbachia-infected males to cause a form of conditional sterility in the targeted populations. A second method known as "Auto Dissemination Augmented by Males" (ADAM) will also be discussed. The ADAM approach employs repeated, inundative introductions of male Aedes mosquitoes to distribute pyriproxyfen as an inhibitor of immature Aedes development. Data from recent laboratory and field trials will be summarized in relation to targeting Aedes mosquitoes as globally invasive pests and medically important vectors.

241 **Evaluation of a Nanoencapsulated Formulation of the Essential Oil Insecticide: BigShot Maxim**

Kai Blore, kblore@amcdfi.org

A bottle bioassay was conducted to assess the efficacy of a non-commercial, experimental formulation of BigShot Maxim distributed by PreVasive USA, LLC. The formulation is a glycerine-based polymer encapsulated cedar oil (15.2% AI by weight) with trace amounts of thyme, cinnamon, peppermint and lemongrass organic oils. Four trials were conducted during this experiment but only the data from Trials 2 – 4 were used. Trial 1 was evaluated across a wider range of concentrations which was used to determine a narrower range for subsequent testing. For the bottle bioassay, 4 concentrations of the test material (1:50, 1:100, 1:150 & 1:200) were prepared via serial dilution with acetone. The interior of each bottle was evenly coated with 1mL of the respective dilution for a total treatment of 13.3, 6.6, 4.4 and 3.3 µg/cm² of AI respectively. A negative control with 1mL of acetone and a positive permethrin (CDC diagnostic dose) was also included. At 1h post-treatment, mortality ranged between 27.3-93.0% while 24h results exceed 95% mortality for all concentrations. Lethal concentration at 1h post-treatment calculated via probit analysis were LC₅₀= 4.9 µg/cm² (4.7 – 5.2 µg/cm² 95% CI; LC₉₀=10.0µg (9.1 – 11.2 µg/cm² 95% CI).

Good Laboratory Practices (GLP) in Mosquito Control Product Evaluation - The Anastasia MCD Experience

Karl Malamud-roam, Vector.Control.Consultants@gmail.com

Most mosquito control programs test and evaluate mosquito control products as part of their routine operations, but few have seriously evaluated their inhouse testing programs. Anastasia Mosquito Control District (AMCD) in Florida is one of the few, and this presentation covers the District's efforts over three years to comply with EPA's formal Good Laboratory Practices (GLP) requirements. Companies that submit mosquito control products to EPA for registration must provide reliable data on the product's safety and efficacy, and EPA requires that these data are collected by GLP-compliant labs to ensure data reliability. Proving GLP compliance is a major task, as EPA's inspectors review test facilities, personnel, equipment, archives, insectary operation, chemical storage, and many other realms. In particular, they demand that all raw data is signed, dated, and retained, and that any GLP study can be fully "reconstructed" from test facility archives. This requires that all significant actions that could impact data quality are documented in formal Standard Operating Procedures (SOPs), that staff is evaluated for competency, that suppliers and equipment are verified, and that an independent Quality Assurance Unit reviews all work by the study team. Finally, any deviations from SOPs or Study Plans must be recorded, investigated, and remediated before a study can be considered GLP-compliant. AMCD conducts a significant number of product evaluation studies each year and decided that the ability to conduct GLP-studies would be a significant addition to the District's capabilities. This presentation, by a GLP consultant that has worked with WHO Collaborating Centers and other mosquito labs around the world, will cover the GLP requirements and EPA's inspection process, and will use AMCD as a case study of a MCD that is adding GLP to its testing and evaluation program.

Behavior/Biology II

Vertebrate Hosts of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* (Diptera: Culicidae) as Potential Vectors of Zika Virus in Florida Tanise Stenn, Karlette J Peck, Glauber Rocha Pereira, Nathan D Burkett-Cadena

tanise.stenn, tanise@ufl.edu, Karlette J Peck, Glauber Rocha Pereira, Nathan D Burkett-Cadena

Zika virus (ZIKV), once considered an obscure pathogen, spread rapidly from 2014 to 2016 to become an internationally notifiable condition of major public health concern. The relative importance of various *Culex* and *Aedes* species mosquitoes (Diptera: Culicidae) in ZIKV transmission is a topic of debate. Quantifying host use is important in determining the vectorial capacity of a mosquito species for transmitting ZIKV in nature. In the United States, few data are available on host use of *Aedes aegypti* L. (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae), confirmed and suspected vectors of ZIKV, respectively. Here, we report results of bloodmeal analysis to quantify host use of confirmed (*Ae. aegypti*) and suspected (*Ae. albopictus* and *Culex quinquefasciatus* Say (Diptera: Culicidae)) vectors of ZIKV in two Florida counties. At an auto salvage yard in Indian River County, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* fed mainly on humans, taking 90.2, 90.8, and 78.6% of bloodmeals from humans, respectively. At a residential area in Martin County, *Ae. aegypti*, *Ae. albopictus* took 61.5 and 66.7% of bloodmeals from humans, higher than *Cx. quinquefasciatus* (11.1%).

Patterns of host use suggest that *Ae. aegypti* and *Ae. albopictus* are the most likely vectors of ZIKV in Florida and that *Cx. quinquefasciatus* would likely play a lesser role in ZIKV transmission in Florida. However, the relative importance of the three species in ZIKV transmission is likely location and population specific. Detailed studies quantifying other parameters of vectorial capacity, including vector competence, are needed in order to determine the actual role for each species in ZIKV transmission.

244 **Studies on effect of *Andrographis paniculata* (Burm.f.) extracts against mosquitoes by using a high throughput screening system**

Patcharawan Sirisopa, golf.patcharawan@gmail.com

Plant repellents are increasingly recognized as a promising and alternate approach of preventing vector-borne illness transmission. *Andrographis paniculata* (Acanthaceae) is an effective natural insect repellent. The objective of this study is to evaluate repellent properties from four active compound (Andrographolide, 14-Deoxy-11,12-didehydroandrographolide, Neoandrographolide and Andrographolide mix with Neoandrographolide) extracted from *A. paniculata* against *Aedes aegypti*, and *Anopheles minimus* by the high-throughput screening system (HITSS). Spatial repellency response, contact irritancy response and toxicity in mosquitoes to all test compounds were observed at 2.5% concentration. The highest contact irritancy response on mosquitoes was from crude extract. While the highest spatial repellency was from andrographolide. Low toxicity was observed in all active ingredients. In conclusion, *A. paniculata* and andrographolide are potentials as active ingredients for mosquito repellents and presents no toxicity.

245 **Effect of Incandescent Light on Collection of West Nile Virus Vectors using CDC Miniature Light Traps in Northern Colorado**

Broox Boze, bboze@vdc.net, Daniel Markowski

To evaluate whether the presence of clear incandescent light was attractive or refractive to host-seeking mosquitoes in northern Colorado, a Bayesian hierarchical model was created to measure differences in trap effectiveness based on presence or absence of phototactic cues. A total of 8 CDC miniature light traps (with and without light) were set weekly across 4 locations in northern Colorado between Weeks 23 and 32 of year 2020. *Culex* mosquitoes (Diptera: Culicidae) accounted for 81% of all collections in this study with two vectors of West Nile virus being represented. The probability of catching both *Culex tarsalis* Coquillett and *Culex pipiens* Linnaeus was reduced when traps were equipped with light, but the difference was not statistically significant for *Culex tarsalis*. The clear reduction in the number of *Culex pipiens* caught when these traps were equipped with light indicate negative phototactic behavior and underestimation with current surveillance strategies. Removal of light from these traps may aid our understanding of these species' distribution within the environment, improve collection efficiency, and help guide implementation of targeted control measures used in public health mosquito control.

246 **Comparison of different larval diets and an ammonia reducing compound on the mass rearing of *Aedes aegypti***

Steven Stenhouse, stenhouse@lcmcd.org, Johanna Bajonero Cuervo, Rachel Morreale, Aaron Lloyd, David Hoel

Lee County Mosquito Control District uses Purina® AquaMax® Fry Powder to feed larval *Aedes aegypti* for mass rearing. In November 2020, differences in granule size of a new batch of fry

powder were noted compared with previously used food. While there was excellent synchronization of pupation with the highest yield of males from the AquaMax® Fry Powder, individuals maintained on this diet had the shortest longevity, lowest pupal size, and smallest wing lengths. Fry powder that matched the previous granule size, as well as another Purina® product, Fry Starter 100, yielded greater pupal sizes, longer survival, and larger wing lengths but both had less pupal synchronization and produced fewer males. Due to these mixed results, it was necessary to reevaluate diets to ensure that we were using the best performing diet. Additionally, during mass rearing of *Ae. aegypti*, an intensely strong smell of ammonia was noted. An ammonia reducer, ProLine® Dry Ammonia Remover, was investigated as a possible additive to the larval rearing trays. We tested Fry Powder, Starter 100, and a combination diet consisting of both feeds. All diets were evaluated with and without ammonia reducer. This study examined the extent to which changing larval diets and the presence of an ammonia-reducing compound could improve larval rearing. Hatching and viability were greatest in treatments that were given a combination diet of Starter 100 and Fry Powder. Females obtained from each treatment were blood fed and allowed to oviposit. There were no differences in eggs per female, but hatch rates were greater among eggs produced by females that had received either the combination diet or Starter 100, without ammonia reducer. The addition of the ammonia reducer, regardless of diet, resulted in reduced adult production. All treatments in which ammonia reducer was administered yielded females with shorter wing lengths.

- 247 **The effects of sub-lethal transfluthrin exposure on *Aedes aegypti* flight and host-seeking behaviors in a chamber with stable airborne concentration gradients**
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Arthropod disease vector control advancements is a safeguard to prevent mosquito-borne disease pandemics. Transfluthrin is currently a popular vaporized pyrethroid used to deter mosquitoes, though there is a dearth of data connecting pyrethroid susceptible-*Aedes* (*Ae.*) *aegypti* behavior to precisely quantified transfluthrin doses. Using a flow- and temperature-controlled flight chamber with clean-air dilution capabilities, we obtained CO₂ concentration gradients in the range of 5-10× and temperature differences of 10-12°C when comparing the chamber inlet to the outlet. This novel chamber was used as a platform to understand mosquito flight and host-seeking behaviors in response to sub-lethal transfluthrin doses, both with and without the presence of host cues: heat, CO₂ and Biogent-Sweetscent™. Our current data indicate that *Ae. aegypti* position in the chamber is not dramatically altered in response to transfluthrin exposure without attractants, while surprisingly more mosquitoes prefer the inlet side of the chamber when transfluthrin exposure occurs in combination with attractants. Further, host-seeking and probing is decreased at the inlet with transfluthrin and attractants present accompanied by increases in lateral and time-in-flight patterns. Our future work with this unique chamber will add active air sampling coupled with thermal desorption-gas chromatography-mass spectrometry to precisely define airborne threshold transfluthrin concentrations that correlate to changes in mosquito flight and host-seeking behaviors.

- 248 ***Culex quinquefasciatus* activity patterns as elucidated by BG-Counters in Iberia Parish, Louisiana**
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Following preliminary studies in 2019, BG-Counters have been deployed regularly by Iberia Parish Mosquito Abatement to monitor mosquito activity patterns. The counters have been incorporated into 2 types of traps: modified gravid traps, baited with fish emulsion, and BG Sentinel 2 traps, baited with carbon dioxide. Research has primarily focused on *Culex quinquefasciatus*. In order to examine activity patterns of host-seeking females of this species, large numbers of larvae and pupae were collected from known breeding sites and placed in an outdoor propagation container at our facility. A BG Sentinel 2 trap, fitted with a BG-Counter and baited with carbon dioxide, was placed very close to this container for 3 days. This process was repeated weekly. Modified gravid traps, fitted with BG-Counters and baited with fish emulsion, were placed at sites of known *Cx. quinquefasciatus* activity in the parish. Activity traces from these gravid traps (catches from which were indeed dominated by *Cx. quinquefasciatus*) show sharp post-sunset and pre-dawn activity peaks. Early evening activity tends to rise sharply soon after sunset, peaking shortly after twilight end, then drops off sharply. BG Sentinel 2 traps near the propagation container were also generally successful in catching primarily *Cx. quinquefasciatus*. Unlike the gravid trap counters, these counters show activity increasing rapidly just after twilight end and reaching a maximum about 30 min thereafter, at the very time the species' activity at the gravid traps is usually declining rapidly. Elevated activity continues throughout the nighttime hours. Somewhat elevated activity also tends to occur in late morning. In both cases the timing of the onset of high activity levels in relation to sunset appears to be quite consistent, independent of temperature or time of year.

250 **The University of Georgia Black Fly Research and Resource Center**
 Elmer Gray, ewgray@uga.edu, Darold Batzer, Danny Mead, Peter Adler

The University of Georgia Black Fly Research and Resource Center is collaborating with the National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) to provide black fly resources to interested researchers. This collaboration supports the world's only black fly colony (*Simulium vittatum* cytospecies IS-7). The black fly colony is a unique, one of a kind, resource that was initiated in 1981 at Cornell University and has been maintained at the University of Georgia since 1999. Black flies require flowing water to complete their life cycle. The colony simulates this environment with 9 aquatic rearing units that create miniature rivers for the larval and pupal stages to develop. All stages of the black fly life cycle are available, live or preserved, as well as total nucleic acid extracts. These materials provide a unique opportunity for research on this important vector group and the biological comparisons that can be conducted with an organism that has been maintained isolated in colonization for forty years. It should be noted that the colony is pathogen free and was genetically characterized about 20 years ago so there is baseline information available. Material from the colony could be used to efficiently conduct vector biology, insect pathology, genetic, predator-prey interactions and a host of other research endeavors. Those interested in receiving material from the black fly colony should work through the BEI Resources website (www.beiresources.com).

Adult Control II

251 **"Lag of the Art" - Historical challenges for US adulticide space sprays and the future of development**
 Jason Clark, jasdclark@yahoo.com, Banu Kesavaraju, Dave Schumacher

Since 1962, the US EPA has registered close to 60 mosquito adulticide space spray products but these only represent 2 distinct modes of action. Challenges of market size, environmental policy, canceled registrations, active ingredient availability, formulation compatibility, manufacturing logistics, product development timelines, resistance development, and mosquito control operational expectations have all contributed to a lag in the development of highly efficacious, user friendly space spray tools for mosquito control programs. A review of how these challenges have historically been approached, are being addressed currently, and recent advancements in research of new space spray tools will be discussed.

253 **The evolution of genetic structure of population of two species of the *Anopheles gambiae* complex using *kdr* gene before and after Indoor Residual spraying in some agroecological zones of Benin**

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Introduction: In Benin, the resistance of *An. gambiae* s.l. mosquitoes to pyrethroids remains a major concern. The development and use of alternative insecticides seems to be the solution. Organophosphates (Actelic@300 CS) are currently the best candidates to meet this challenge. The present study aims to compare the genetic structure of vector populations of two species of the *An. gambiae* s.l. complex through the L1014F resistance allele of the *Kdr* gene during two periods marked by characteristic environments to better understand its impact on the dynamics and biology of these organisms.

Methods: Eight locations in three agro-ecological zones, one of which is a control, were studied. Larval surveys were carried out during the rainy seasons of May to July 2016 for the pre-IRS period and May to November 2018 for the post-IRS period. Larvae were reared at the insectarium of the Centre de Recherche Entomologique de Cotonou. The adult females obtained were identified morphologically and by molecular approaches. Thanks to the L1014F and L1014L alleles of *Kdr*, the genetic structure of the populations at various hierarchical levels could be determined.

Results: Molecular analysis revealed three vector species of the *An. gambiae* complex both before and after IRS, two of which are in the majority. These were 171 *An. coluzzii*, 297 *An. gambiae* s.s., 11 *An. arabiensis* and 211 *An. coluzzii*, 256 *An. gambiae* s.s., 8 *An. arabiensis* respectively out of 479 sampled before treatment and 475 sampled after IRS. In both *An. gambiae* s.s. and *An. coluzzii*, the gene frequency of L1014F was found to increase significantly following treatment, approaching the limit of fixation in some populations. A deficit of heterozygosity is widespread with values of the indices, *F_{ST}*, *F_{SC}* and *F_{CT}*, expressing little or no differentiation within and between the defined populations.

Conclusions: IRS has more favoured the selection and spread of the L1014F resistance allele of the *Kdr* gene. The apparent adaptation of *An. coluzzii* to polluted areas would be a factor in its proliferation to the detriment of its twin sister *An. gambiae* s.s. in IRS areas. The genetic structuring of the populations, whatever the species, remains almost the same despite the treatment.

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Mosquito Magnet Executive Trapping for Neighborhood protection from Mosquitoes

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Commercial mosquito traps powered by electricity or propane are advertised to protect homeowners from biting insects. The Mosquito Magnet Executive (MME) trap claims to protect an acre of land from biting mosquitoes and is sold to homeowners for that purpose. A 2-year study looking at the effect of placing 33 MME traps in a neighborhood on Sanibel Island, 1 mile off the west coast of Lee County, Florida, was started in early 2021. Sanibel Island is renowned for the tremendous number of saltmarsh mosquitoes it produces in its many marshes, mangrove fringes and forests. *Aedes taeniorhynchus*, the Black Saltmarsh Mosquito, breeds as heavily here as anywhere in the state, including the Everglades. These high populations drew researchers from state entomological agencies in the 1950s to study its biology in an attempt learn how to control them. Among their findings are that as many as 39,000 eggs are laid per square foot of soil. The neighborhood that was chosen for this study was divided into 3 sections that are each about 33 acres in size. The middle section served as the control, and the two ends of the neighborhood as treatments. In year 1, 3 CDC traps were set in each section and monitored twice weekly starting in May 2021. About 2 weeks after the surveillance traps were started, the MME traps were placed in the West section and started. All traps utilized MM Octenol as an added attractant. Collections from the MME traps were pooled into weekly collections. The end point is reduction in CDC trap numbers, so while the MM species collected is of interest, it is not the most important factor in this study. Preliminary findings from year one of this study will be presented.

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Community Knowledge, Attitude and Practices on Malaria Vector Control Strategies in Lagos State, South-West Nigeria

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Malaria is a leading public health challenge causing mortality and morbidity in sub-Saharan Africa. Prominent malaria vector control methods employed in sub-Saharan Africa include Long Lasting Insecticide Nets (LLINs) and Indoor Residual spraying (IRS). This study investigated knowledge, attitude and practices (KAP) of malaria vector control methods in Lagos, South-West Nigeria. Structured questionnaires were employed for the cross-sectional survey which was carried out between May and August 2018. Multi-stage sampling technique was used to select Lagos Mainland, Kosofe, and Ojo local government areas (LGAs). Five hundred and twenty questionnaires were used for the study. Data were analyzed for descriptive statistics, whereas χ^2 was used to determine influence of respondents' LGA, level of education and type of dwelling on respondents' attitude and practice. Respondents' LGAs have no significant impact on attitude and practice to malaria vector control methods. However, 'level of education' as well as 'type of dwelling structure' impacted significantly on some practices and attitude. Basically, IRS is the major tool employed in malaria vector control, but sometimes it is used in combination with other methods. A good number of residents also use LLINs. 'Choice of method' employed is mainly based on the effectiveness of method. General perception about LLINs and IRS is that they are effective, cheap and safer. Considering the widespread use of IRS and LLINs for malaria vector control in Lagos, implementation of malaria control programs should consider KAP to these two strategies.

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Survey of United States mosquito control programs reveals opportunities to improve the operational value of Centers for Disease Control and Prevention bottle bioassays

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Centers for Disease Control and Prevention (CDC) bottle bioassays are widely used by mosquito control programs (MCPs) to assess insecticide resistance (IR). If this screening tool indicates IR in mosquitoes, additional testing is necessary to further verify and evaluate possible reasons for IR. The aim of this study was to assess knowledge and practices of MCPs in the United States (US) on bottle bioassay usage for tracking IR and how such data are used to inform intervention decisions. A survey of IR knowledge and practices was conducted and, although varying levels of bottle bioassay usage were discovered, widespread interest was evident. 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 MCPs. Consequently, the analysis of survey responses identified opportunities to improve the operational value of CDC bottle bioassays, improve the detection of IR, and understand the impact of IR on mosquito control measures. For example, clear universal guidelines should be developed for the practical use of bottle bioassay data as it relates to operational impacts in MCPs. Although many respondents indicated that route of exposure impacts mosquito mortality (68% of respondents) and/or development of IR (42% of respondents), many respondents were unsure if route of exposure impacted mosquito mortality (27% of respondents) or IR (49% of respondents). 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. When the above indicated improvements to IR monitoring are incorporated into MCPs, it will improve the outcome of effective IR management.

257 **Assessing insecticide resistance and potency directly on the mosquito central nervous system: a novel method**

Edmund Norris, Edmund.Norris@usda.gov, Jeffrey Bloomquist

Resistance to currently utilized chemical insecticides and public push back against new insecticide registrations represent significant threats to public health and food security worldwide. Better understanding the neurophysiological effects of currently available and candidate insecticides is crucial in evaluating cross-resistance in wild arthropod populations and the utility of new compounds to control pests. Here we highlight a novel method of recording nerve firing from the central nervous system of fourth instar larvae from two medically relevant mosquito species, *Aedes aegypti* and *Anopheles gambiae*. In short, 1) mosquitoes were carefully dissected to provide access to the ventral nerve cord, 2) the nerve cord was then severed between the 2nd and 3rd abdominal ganglion, and 3) a recording suction electrode was connected directly to the descending connective in a novel way to record nerve firing in the ventral nerve cord. This method provided a high signal-to-noise ratio compared to previously attempted protocols needed to characterize drug effects. Using this method, we report the effects of various neuroactive compounds, ranging from neurotransmitters to insecticides. We also explore the potential of characterizing insecticide resistance using this new preparation at the level of the nerve by screening on both larvae from pyrethroid-susceptible and pyrethroid-resistant populations.

258 **Toxicity of Different Groups of Insecticides and Determination of Resistance in *Aedes albopictus* from Different Habitats**

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Huge amount of insecticides are used for the control of agricultural pests and house hold pests like mosquitoes with over and under doses in Punjab, Pakistan. Moreover, after dengue

epidemics during 2010, insecticides in huge amount with high doses were sprayed in major cities of the Punjab to control mosquito. This also resulted in insecticidal resistance in mosquitoes. So, the present study was undertaken. Mosquitocidal assays were evaluated against larvae and adults after 24 h. Larvicidal LC50 of temephos ranged 0.0075 to 0.422 $\mu\text{g gm}^{-1}$. In case of adulticides, three groups of insecticides used on papers to twelve different populations collected from populated, agricultural and industrial areas of Lahore (LHR), Rawalpindi (RWP), Sialkot (SKT) and Faisalabad (FSD). Among insecticide group, synthetic pyrethroids were recorded as highly potent and extremely toxic at lowest concentration followed by Organophosphates (OP's) and carbamates. However, Synthetic pyrethroid group included Deltamethrin, recorded as highly potent (0.483 – 9.245 $\mu\text{g gm}^{-1}$) followed by cypermethrin (1.831 – 32.189 $\mu\text{g gm}^{-1}$) and permethrin (5.05 – 100.453 $\mu\text{g gm}^{-1}$). OP's group comprised of Pirimphosmethyl found highly toxic (12.123 – 311.712 $\mu\text{g gm}^{-1}$) followed by Malathion (20.961 – 612.525 $\mu\text{g gm}^{-1}$) and Fenitrothion (36.12 – 601.413 $\mu\text{g gm}^{-1}$). Carbamate group included Bendiocarb (70.08 – 6154.79 $\mu\text{g gm}^{-1}$). The Chi square value showed no heterogeneity in all experiments. The results also indicated that LHR population was highly resistant followed by RWP, SKT and FSD population, moreover, mosquito populations from agricultural areas were more resistant than populated and industrial areas. Biochemical analysis showed the elevated activity of enzymes (esterases, mixed function oxidases, glutathione S transferase and acetyl-cholinesterase) in resistant populations. It was concluded that injudicious application of chemicals in an area, initiated risks of resistance, reappearance and resurgence of certain mosquitoes. Further, research must be needed to identify health, environmental risks and devise an effective program by using selective and specific insecticides.

Latin American Student Competition Symposium

261 **Intestinal microbiota in *Aedes aegypti* larvae (Linnaeus, 1762) and the relationship with the susceptibility to insecticides temephos and permethrin.**

Heriberto M. Villegas-Ramirez, h.villegasrmz28@gmail.com, Gustavo Ponce, Adriana Flores, Karina Villanueva, Iram Rodriguez, Idelfonso Fernandez

There are about 3500 species of mosquitoes belonging to the Culicidae family, within which 225 species belonging to 21 genera are registered in Mexico, some of them of veterinary medical importance due to their ability to transmit various pathogens to humans and animals.

The over human population, the effect of climate change among other factors, have favored the distribution of *Ae. aegypti*, for which they have applied various strategies for their control, among which is chemical control, a strategy whose implementation has caused an excessive use of insecticides, generating the phenomenon of resistance to them.

The results obtained showed that the Guadalupe population presents a CL₅₀: 0.022 ppm and a CL₉₉: 0.114 ppm for temephos, for cis-permethrin a CL₅₀: 0.071 ppm and a CL₉₉: 3.882 ppm. In the susceptible New Orleans (NO) population, a CL₅₀: 0.003 ppm, a CL₉₉: 0.012 ppm for temephos was obtained and in cis-permethrin a CL₅₀: 0.001 ppm and a CL₉₉: 0.067 ppm were obtained. With these results the Resistance reason (RRCL₅₀) was determined, which for temephos presents a tolerance of RRCL₅₀:: 7.33 and presenting a cis-permethrin resistance a RRCL₅₀: 71.

In relation to the microbial communities at the phylum level, they indicate that Proteobacteria is the one with the highest abundance with 66.4%, Bacteroidetes with 19.8%, Actinobacteria with 11.4% and the rest represents less than 5% of the abundance of the 6 treatments. Likewise, the values obtained at the reproduction family level that for live temephos treatments, the bacteria with the highest abundance was Comamonadaceae with 48.33%, for dead temephos it was Microbacteriaceae with 37.17%, for live cis-permethrin it was Comamonadaceae with 30.33%, in cis- permethrin killed with 36.00%, for New Orleans it was Flavobacteriaceae with 41.30% and finally the field treatment, with Rhodocyclaceae with 32.23%.

At the species level, bacteria are not shown for both the live and dead treatments of both insecticides, which can influence greater or lesser susceptibility, differences in the number of species are shown, being the field the one that presented the greatest diversity with 301, being Rhodocyclaceae_Na_sp with 31.93% the most abundant, the New Orleans treatment was the least diverse with 36, being Elizabethkingia meningoseptica the most abundant with 38.12%.

The alpha diversity indices, such as the Shannon, Chao1 and Fisher indices, indicate that the treatment with the most diversity is field, whereas for Simpson, it was the live cis-permethrin treatment that presented the greatest diversity, finally, by of the beta index, Bray Curtis, there was similarity between the live and dead treatments of temephos, on the other hand, for cis-permethrin, both alive and dead, there was dissimilarity. In conclusion, the intestinal microbiota in larvae of Ae. aegypti does not influence a lower or higher susceptibility to insecticides.

262 **In vivo characterization of three knockdown resistance alleles in pyrethroid resistant-congenic strains of Aedes aegypti**

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Aedes aegypti is the primary vector of important arboviral diseases and pyrethroids are the most common class of insecticides to control adult mosquitoes. The presence of knockdown resistance mutations (kdr) mutations confer resistance to pyrethroids. Our previous results found higher resistance levels to the triple-mutation kdr allele relative to the single-mutation allele. Thus, we used functional genetic approaches to evaluate the role in resistance to pyrethroids of the double-mutation alleles derived from the triple-mutation allele.

263 **Hotspots of pyrethroid and kdr resistance in Aedes aegypti from Mexico**

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The spatial distribution pattern of the frequency of kdr resistance to pyrethroids in populations of Aedes aegypti from Mexico was determined. Mosquito populations were collected from 45 locations distributed in 15 states. Bioassays were performed with diagnostic doses (DD) of the insecticides permethrin (pyrethroid type I) and deltamethrin (pyrethroid type II) in each population, to determine the frequency of resistance. Subsequently, the results of the bioassays were correlated with the frequencies of the V410L, V1016I, and F1534C mutations obtained by quantitative PCR. All populations of Ae. aegypti showed high frequency of resistance to permethrin, with the greatest foci of resistance in the populations of the states of Veracruz, Tabasco, Guerrero and Sinaloa. Forty percent of the populations had high frequency of resistance to deltamethrin, 28% with moderate and 32% were

susceptible, with the greatest focus of resistance in the populations of the state of Tabasco. The most frequent genotype was VL / VI / CC followed by triple resistant LL / II / CC. The foci of greater resistance to permethrin were significantly associated with the triple resistant genotype (LL / II / CC), however, for deltamethrin, the genotypes with the highest frequency were triple resistant (LL / II / CC) and double heterozygous for 410 and 1016 and resistant for 1534 (VL / VI / CC). Our results show the presence of *kdr* resistance foci associated with resistance to type I and II pyrethroids in *Ae. aegypti* in Mexico.

264 **Feeding status and host preference of *Anopheles* species from malaria endemic areas of west and northwest Colombia**

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Malaria parasites are transmitted by *Anopheles* female mosquitoes which bite humans to obtain blood. In Colombia, the Bajo Cauca and Pacific regions are currently the most malaria endemic areas of the country. To determine *Anopheles* vectorial capacity, it is necessary to evaluate feeding behavior, which is influenced by vector intrinsic or extrinsic factors. Therefore, the aim of this work was to identify blood feeding sources and host preferences for *Anopheles* species of endemic localities in the Bajo Cauca and Pacific regions. Mosquitoes were collected using barrier-screens, for four days, between 18:00 and 24:00 hours, localities were visited twice over the years 2018-2021. During collections, a census of the animals present at the sampling sites was conducted within a radius of 250 meters, every day. Mosquitoes were identified by available morphological keys and molecularly confirmed by PCR-RFLP-ITS2. Blood feeding sources were detected by PCR with primers targeting the MT-CYB and COI genes for vertebrates in DNA extracted from mosquito midguts. The Human Blood Index and Forage Index were estimated to determine female's blood feeding preference. In total, 524 *Anopheles* specimens were collected. *Anopheles* (Nys.) *darlingi* Root, 1926, was the most abundant species in the Bajo Cauca and *Anopheles* (Nys.) *nuneztovari* Gabaldón, 1940, in the Pacific localities. The highest human blood index was detected for the main vectors *An. nuneztovari* (57.1%) and *An. darlingi* (40%); however, *An. nuneztovari* showed preference for feeding on dog blood and *An. darlingi* on pig, dog and Galliforms blood, rather than humans. These results provide valuable information to orientate effective control interventions directed to minimize human-vector contact, in accordance with the *Anopheles* species feeding behavior.

Latin American Symposium

265 **Entomological survey to determine the role of cisterns in the production of *Aedes aegypti* in the US Virgin Islands**

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Aedes aegypti is commonly found in urban and suburban areas in the US Virgin Islands (USVI) and is the vector of dengue, Zika, and chikungunya viruses. Given the limited potable water supply in the islands, most residents use cisterns to collect rainwater and store it for their everyday needs. A survey conducted in 2019 found that 46% of the cisterns contained mosquitoes (unpublished). A follow-up representative entomological survey was designed to determine their role as producers of

Ae. aegypti mosquitoes as well as understanding the factors that influence productivity. Three funnel traps were installed inside each cistern sampled, and exit traps were installed in the cisterns' intake spouts and overflow pipes, when possible. Cistern physical and chemical characteristics were also recorded.

A total of 1,769 households were visited with a response rate of 25%. Of those, only 76% of cisterns met protocol criteria, resulting in 342 cisterns sampled.

Preliminary results indicate that 49.1% of the cisterns surveyed were positive for immature mosquitoes. When divided by district, a higher percentage was observed for St. Thomas/St. John district (57.3%) as compared with St. Croix (40.9%).

However, on average, only 5.8% of the exit traps captured adult mosquitoes, with St. Thomas/St. John district showing a higher capture than St. Croix (6.4% versus 5.3%, respectively). Most mosquitoes collected (81.1%) were identified as *Ae. aegypti*.

Based on the results from this study, vector management strategies will be developed to reduce the impact of cisterns as breeding sites for *Ae. aegypti*.

266 **Anopheline species complex of Venezuela and implications for their control**
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Understanding the taxonomic status of anophelines involved in the transmission of malaria parasites, their distribution and bionomics is essential for the implementation of effective vector control programs. The use of molecular tools has shown the presence of at least six species complex within the Neotropical vectors of malaria parasites. In Venezuela, so far four species complexes have been studied: *Albitarsis*, *Nuneztovari*, *Oswaldoi* and *Triannulatus*. The *Albitarsis* complex is represented by the mitochondrial lineages of *Anopheles albitarsis* I and *An. albitarsis* F, the latter is a confirmed vector of *Plasmodium vivax* and *P. falciparum*, mainly in the hot spot of gold mining areas of southern Venezuela together with *An. darlingi*, *An. nuneztovari* s.l., and *An. oswaldoi* B. The complex *Nuneztovari* is represented by *An. nuneztovari* s.s. in western Venezuela, a confirmed vector, and *An. goeldii*, from malaria endemic areas in southern Venezuela. Both species showed distinctive biting patterns which must determine the most effective control tool to be used. *Anopheles oswaldoi* B and *An. triannulatus* s.s. have been identified only in southern Venezuela. Further studies on population genetics are required to elucidate the species present in Venezuela, their distribution and contribute to the formal description of species such as *An. albitarsis* I, *An. albitarsis* F and *An. oswaldoi* B.

267 **Identifying male seminal fluid proteins required for reproduction in the dengue vector mosquito *Aedes aegypti*.**
catalina alfonso, catalfonso@gmail.com, Sara Villa-Arias, Frank W. Avila

Aedes aegypti males transfer sperm and seminal fluid proteins (SFPs) to the female during mating. As in other insects, SFPs induce numerous behavioral and physiological changes in mated *Ae. aegypti* females, collectively known as the female post mating response, which includes an increase in longevity, increases in the rate of egg development and the induction of egg laying, and a decrease in sexual receptivity. In addition, SFPs are likely to mediate the storage of sperm in the female reproductive tract, as is observed in other insects. Despite the importance of reproduction in vectorial capacity and vector control, the seminal proteins that elicit any of the post-mating changes in female

behavior and/or physiology have yet to be identified. The goal of this study was to determine the SFPs that participate in sperm storage and/or ovulation and egg-laying. As SFPs that mediate these processes associate with sperm, the sperm storage organs, and/or the oviduct in the model *Drosophila melanogaster*, our aim was to determine *Ae. aegypti* SFPs that localize to the spermathecae (the sperm storage organs in *Ae. aegypti*) and/or the oviduct. We used mass spectrometry of protein extracts from spermathecal and oviductal tissue of N15-labeled females after mating with non-labeled males, which allowed us to identify male-derived seminal proteins that localize to these tissues. This project is a first step in identifying SFPs that mediate female post-mating responses in *Ae. aegypti* vector mosquitoes.

268 **Analysis and comparison of microbiota in *Aedes aegypti* larvae in different types of artificial breeding sites.**

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The microbiota in mosquitoes has played a fundamental role in the transmission of diseases, so new control strategies based on the manipulation of the host microbiota are being sought. For the proper design of these new strategies, it is necessary to start by knowing the composition of the microbiota in these vectors of diseases transmission.

Aedes aegypti breeding habitats harbor a diversified set of natural microbiota, so this research will look for the types of artificial breeding sites of *Ae. aegypti* to subsequently characterize by high-throughput sequencing of the region to amplify the hypervariable regions of the 16S rRNA gene and analyze the composition and its influence on these larval habitats.

In relation to the microbial communities at the phylum level, Proteobacteria is the one with the highest abundance with 46.07%, the second phylum was Bacteroidetes with 18.59% and the third phylum with an abundance of 14.67% was Firmicutes. The phylum observed in lower abundance are Fibrobacteres with 0.03%, Nitrospirae with 0.04% and Chlamydiae with 0.07% concluding that the larvae acquired their microbiota from the habitats in which they are found.

The alpha diversity indexes, Shannon's and Simpson's indexes, indicated that the tire hatchery type has greater diversity compared to the other groups. The results of the Chao1 and Fisher indexes indicate that the metal container hatchery has greater species diversity. The Bray-Curtis beta diversity index shows that there is dissimilarity between the groups analyzed. On the other hand, the Hutcheson's t-test indicates that there is no significant difference ($P < 0.05$) between the hatchery treatments analyzed. Therefore, it can be concluded that the type of habitat in which the larvae are located influences their microbial composition.

269 **Blood meal of domiciliary populations of culicids in metropolitan area of Monterrey, Nuevo León**

Damaris Alejandra Luis Solis, damaris.luis95@gmail.com

Mosquito blood meal patterns provide information on the ecological transmission cycles pathogens, help determine vector status in the maintenance and epidemic transmission of arboviruses, and lead to more efficient disease and vector control measures for the benefit of health.

To determine the blood feeding preference of culicids in Nuevo León, a mosquito collection was carried out using BG-GAT[®] (Biogents) and electric aspirators, the study area was chosen according to the

Methodological Guide for Surveillance Entomological with CENAPRECE Ovitrap and at the time of placing the traps a survey was carried out to determine the number of possible hosts, the monitoring of traps was carried out once a week and the females and species were identified using single-access key and individually stored at -80 ° C.

DNA was extracted with the salt technique by Coen (1982) and PCR was performed with DNA extracted from blood-fed mosquitoes using vertebrate-specific cytochrome b primers. The data analysis was carried out with the R language.

In selected urban areas, the possible hosts that were found were 45% birds which belong to the families Columbidae, Icteridae and Passeridae 39% humans, 11% dogs and 5% cats. Within this area, 350 females have been collected that are between 96% of *Aedes. aegypti* 3% *Culex. quinquefasciatus* and 1% *Aedes albopictus*. And from the partial PCR analyzes it was obtained that 13% belong to humans, 41% dogs, 34% birds and 12% cats.

Legislative and Regulatory - Working Across the Aisle Symposium I

270 Legislative and Regulatory Symposium - working across the aisle

Angela Beehler, angela@mosquitocontrol.org

The L&R Symposium is taking on a new format. It is important for AMCA members to hear from the federal employees who create the regulations and the advocates who help influence laws, so each issue discussed during the L&R Symposium will have multiple speakers representing different agencies, missions, and viewpoints. Not all questions will be answered, but connections will be made, and all sides will be considered.

271 Multiagency cooperative approach to the Endangered Species Act

Mark Clifton, mclifton@nsmad.com, Paul Whatling, Susan Jennings, Marah Clark

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 goal of protecting endangered species. In 2013 the National Academy of Sciences outlined an approach for pesticide registration cooperation which was adopted by the USEPA and USFWS. In 2018, this new consultation and evaluation process was utilized for the first time to evaluate Malathion, Chlorpyrifos and Diazinon and resulted in a series of Biological Opinions (BiOP). The results of the consultation between the EPA and USFWS have demonstrated shortcomings in the assessments of impacts to endangered species for mosquito control products. Product usage patterns, application timing, and application rate continue to be assessed on a worst-case scenario basis. In addition, it has been recognized that agricultural models of product deposition are not accurately capturing real-world deposition patterns. The new interagency cooperation process has clearly demonstrated two important needs to ensure future adult control products are assessed accurately: 1) the development of validated deposition models specifically for mosquito control and 2) accurate and comprehensive treatment information databases that would provide information on end-user application patterns. Together these initiatives would support an understanding of the nuances of mosquito control applications and improve the ESA consultation process.

- 272 **Opportunities to validate deposition and drift models for public health applications**
Dave Brown, dabrownsoj@gmail.com, Karen Larson, Susan Jennings, Mark Latham

Ultra-Low Volume public health pesticide applications are unique in that they welcome drift and reject deposition. Absent of concrete data, pesticide risk assessments rely on modeling to estimate the potential impacts products will have on people and the environment. Agricultural models do not accurately depict real-world mosquito control operations, yet this is the best science available, therefore it is used by federal agencies when making decisions that will impact public health applications for the next 15 years. The Legislative and Regulatory Symposium will highlight the need for modeling specific to mosquito control applications. In order to accomplish this task, AMCA is collaborating with federal agencies, registrants, scientist and end-users. This effort will require funding, transparency, and strong leadership, but doing nothing is not an option. “Working across the aisle” not only applies to political parties; for public health mosquito control to survive the current regulatory climate, we must be willing to engage with partners and adversaries to promote sound science, and follow best management practices to ensure that our operations are beyond reproach.

Legislative and Regulatory - Working Across the Aisle Symposium II

- 273 **Reflections and lessons learned from implementing a portfolio-wide packaging transition**
Laetitia Leroy, lleroy@clarke.com

After U.S. EPA testing revealed the potential for fluorinated packaging to be a source of PFAS contamination in liquid pesticides, product manufacturers using fluorinated packaging were encouraged to explore suitable container alternatives that would mitigate this contamination concern. This session will present one registrant’s stewardship experience with eliminating fluorinated packaging from its portfolio, sourcing suitable packaging alternatives, and securing EPA approval to distribute its products in these alternative containers. The goal of this session is to offer process recommendations and best practices for implementing packaging changes that meet regulatory expectations for PFAS-free pesticide packaging, and reinforce the related stewardship obligations of pesticide manufacturers when tackling this work.

- 274 **Unmanned Aerial Systems (UAS) mosquito control application regulatory update**
Joel Buettner, , Peter Brake, Jonathan Rupprecht

Public health mosquito control applications from UAS are expanding the ability of mosquito control programs to respond to early detection of mosquitoes and disease and prevent mosquito problems including vector borne disease by providing rapid, focused, and effective adult mosquito control applications. Many agencies nationwide are operating UAS in a way that limits the environmental footprint of applications and provides a new tool in combating mosquitoes and vector borne disease. In conversations with EPA and other regulators, the use of mosquito control larvicides from UAS seems to be generally well accepted due to the very low risk profile of those materials and the relative ease at which current UAS spray systems are able to deliver low volume liquid and granular products according to existing label language. Mosquito adulticide, however, poses a greater regulatory challenge due to the materials involved and the necessary use of drift inherent in a successful mosquito

adulticide space-spray application. Technological development of UAS-based spray systems is underway; however, most spray systems are designed to provide a more generic agricultural application which works for mosquito larvicides in most cases, but does not meet requirements for most mosquito adulticides. The EPA and other regulators have suggested that a mosquito adulticide could serve as a test case to evaluate the necessary data for a UAS specific label. Mosquito control agencies, AMCA, researchers, and industry could view this as an opportunity to promote the development and testing of spray technology that meets current label requirements and efficacy goals adult mosquito control, support research into developing necessary drift models which the EPA requires to evaluate aerial pesticide applications, and provide field data. As UAS for mosquito control continues to grow, we are in a position to develop mosquito control-specific standards of operation that would assist regulators of pesticides and aviation, to better understand mosquito control needs, risks, and risk mitigations that mosquito control agencies incorporate into their programs.

276 **Strategy for vector-borne disease preparedness and response**
Gary Goodman

The AMCA is committed to establishing and maintaining adequate federal funding mechanisms that enhance the ability of public health agencies to strengthen epidemiology, laboratory, and health information systems for the detection and responses to known and emerging infectious disease threats. Working in collaboration with other public health agencies and the CDC is necessary to identify opportunities and educate our lawmakers of the long-term importance of these programs. Supporting the CDC's vision of a national strategy is imperative toward maintaining and growing the nations protection against vector borne diseases. There is an immediate need to advance innovation and discovery of cutting-edge diagnostic tools, identification of new and emerging vectors and diseases, and research and development to foster new vector control technologies in addition to building new comprehensive vector control programs and to support existing state, county, district or municipal mosquito control programs in their operations. These needs can only be met with the expansion of public health infrastructure and laboratory building to support a myriad of arbovirus-related programs and activities at the federal level. Ensuring the health of Americans is a federal imperative, and these small preventative investments are vastly preferable to the enormous health care costs required after large vector-borne disease outbreaks. Establishing sustainable training, research and suppression programs for vector-borne disease surveillance and control will ensure a robust capacity to mitigate the impacts of not only current threats but also dangerous exotic viruses yet to reach our shores.

Poster Session

P-1 **Pilot trial using mass field-releases of sterile males produced with the incompatible and sterile insect techniques as part of integrated *Aedes aegypti* control in Mexico**
Abdiel Martin-Park, ampark27@gmail.com, Azael Che-Mendoza, Yamili Contreras-Perera, Silvia Pérez-Carillo, Henry Puerta-Guardo, Josué Villegas-Chim, Hugo Delfín-González, Jorge Palacio-Vargas, Fabián Correa-Morales, Norma Pavia-Ruz, Dongjing Zhang, Gonzalo Vázquez-Prokopec, Zhiyong Xi, Pablo Manrique-Saide

The combination of Wolbachia-based incompatible insect technique (IIT) and radiation-based sterile insect technique (SIT) can be used for population suppression of *Aedes aegypti*. Our main

objective was to evaluate whether open-field mass-releases of wAlbB-infected *Ae. aegypti* males, as part of an Integrated Vector Management (IVM) plan lead by the Mexican Ministry of Health, could suppress natural populations of *Ae. aegypti* in urbanized settings in south Mexico. We implemented a controlled before-and-after quasi-experimental study in two suburban localities of Yucatan (Mexico): San Pedro Chimay, which received IIT-SIT, and San Antonio Tahdzibichen used as control. Release of wAlbB *Ae. aegypti* males at SPC extended for 6 months (July-December 2019), covering the period of higher *Ae. aegypti* abundance. Entomological indicators included egg hatching rates and outdoor/indoor adult females collected at the release and control sites. Approximately 1,270,000 lab-produced wAlbB-infected *Ae. aegypti* males were released in the 50-ha treatment area. The efficacy of IIT-SIT in suppressing indoor female *Ae. aegypti* density (quantified from a generalized linear mixed model showing a statistically significant reduction in treatment versus control areas) was 90.9% a month after initiation of the suppression phase, 47.7% two months after (when number of released males was reduced to match local abundance), 61.4% four months after (when normal 10:1 ratio of releases was re-established), 88.4% five months after and 89.4% at six months after the initiation of the suppression phase. A proportional, but lower, reduction in outdoor female *Ae. aegypti* was also quantified (range, 50.0-75.2% suppression).

P-2 Development of mosquito control action thresholds using historical mosquito surveillance and climate data

Madeline Steck, msteck@amcdf.org, Vindyha Aryaprema, Steve Peper, Whitney Qualls, Kristopher Arheart

The Armed Forces Pest Management Board (AFPMB) is responsible for guiding US military vector control operations and has recognized the need to advance action threshold development strategies in operational regions outside of the US. The Anastasia Mosquito Control District (AMCD) has curated an extensive record database of surveillance programs and operational control activities since 2004. These data alongside previously identified climate predictors were used to construct proactive threshold models for initiating surveillance and control of *Aedes*, *Culex*, and *Anopheles* vector mosquitoes. Species counts (*Culex nigrapalpis*, *Culex quinquefasciatus*, *Aedes albopictus*, *Aedes aegypti*, *Aedes atlanticus*, *Aedes infirmatus*, *Aedes sollicitans*, *Aedes taeniorhyncus*, *Anopheles crucians*) were pulled from CDC light trap (2004-2019) and BG trap (2014-2019) collection records. Weekly climate measurements of temperature, rainfall, and relative humidity were lagged by one to three weeks. Models and test parameters were optimized for species and genera combinations using a generalized linear model approach. Preliminary models and climate thresholds were constructed for container *Aedes* (*Ae. aegypti*, *Ae. albopictus*), standing water *Culex* (*Cx. nigrapalpis*, *Cx. quinquefasciatus*), floodwater *Aedes* (*Ae. atlanticus*, *Ae. infirmatus*), saltmarsh *Aedes* (*Ae. taeniorhyncus*, *Ae. sollicitans*), and a combined All mosquito species group. Minimum temperature, maximum temperature, average temperature, relative humidity, and total rainfall were significant at varying lag times for separate genera. Rainfall at a two-week lag is the most commonly significant parameter in these initial models. Model development is still in progress and next steps include validation with AMCD's 2020 and 2021 surveillance data.

P-3 Pyrethrum resistance in *Culex* mosquitoes in Northern Tulare County, California

Javier Valdivias, jvaldivias@deltavcd.com, Andrea Troupin, Crystal Grippin, Mark Nakata, Mir Bear-Johnson, Mustapha Debboun

Adulticide applications are generally used to reduce the public health risk to residents by reducing large mosquito populations or targeting areas with increased West Nile virus activity in mosquitoes. The Delta Mosquito and Vector Control District (DMVCD) has relied on the use of pyrethrum-based pesticides to reduce adult mosquitoes in areas of high mosquito-borne disease risk for several years. This study was conducted to identify areas of insecticide resistance in rural agricultural and urban *Culex* species mosquito populations within the DMVCD. Egg rafts were collected by placing trays with alfalfa-based infusion overnight in each of the DMVCD cities. Adults were hatched in the insectary where the CDC bottle bioassay was performed. Resistance to pyrethrum was observed in rural agricultural *Culex quinquefasciatus* Say populations. Identifying insecticide resistance in different mosquito populations allows mosquito and vector control agencies to optimize their pesticide application regimes in order to reduce mosquito abundance and disease transmission rates.

P-4 Determining a diagnostic dose of pirimiphos-methyl for *Aedes aegypti* using treated bottles

Gabriela González-Olvera, gabygzzo@gmail.com, Rita L. Vizcaino-Cobarrus, Alicia Méndez-Manzanero, Anuar Medina-Barreiro, Azael Che-Mendoza, Oscar David-Kirstein, Pablo Manrique-Saide, Gonzalo Vázquez-Prokopec, Audrey E. Lenhart

There is a pressing need to develop innovative strategies for the control of diseases transmitted by *Aedes aegypti*. The modification of indoor residual spraying to target *Ae. aegypti* is one such strategy. A clinical trial quantifying the epidemiological impact of targeted indoor residual spraying (TIRS) for *Ae. aegypti* control is using a product with pirimiphos-methyl as the active ingredient in the city of Mérida, Mexico. In order to monitor the susceptibility of the local *Ae. aegypti* populations over the course of the trial, we determined a diagnostic dose for pirimiphos-methyl using the Centers for Disease Control and Prevention (CDC) bottle assay method. Two independent laboratories tested a series of 8 concentrations of pirimiphos-methyl eliciting a range of mortality between 0% and 100% in an insecticide-susceptible reference strain of *Ae. aegypti* (Rockefeller). The results suggested a diagnostic dose of 25 µg/ml with a diagnostic time of 30 minutes. This diagnostic dose was used to screen 16 field populations of *Ae. aegypti* (~ 2000 mosquitoes) prior to the implementation of TIRS, with susceptibility reported in all populations at baseline. This diagnostic dose of pirimiphos-methyl will be used to monitor pirimiphos-methyl susceptibility in *Ae. aegypti* throughout the course of the trial.

P-5 Field observations of invasive species *Aedes japonicus* and larval contemporaries in Escambia County, Florida

Michael T. Riles, michael@pcbeachmosquito.com, Dan Killingsworth, Corey Day

The Asian bush mosquito, *Aedes japonicus*, is an invasive species that is well established in North America and Europe. Though it is considered a temperate species, we have observed an established population of *Ae. japonicus* in the subtropical climate of northwestern Florida. To evaluate the temporal patterns of *Ae. japonicus* abundance, mosquito larvae were collected from 15 artificial containers in Escambia County, FL, from August 2019 to July 2020, with the prediction that *Ae. japonicus* abundance would peak in the winter months and decline with increasing ambient temperatures. *Aedes japonicus* larvae were collected in low abundances during each month except for February (n = 51), with no clear temporal patterns of abundance. Larval contemporaries belonging to other species were considered in sampling of containers and were also cataloged. We demonstrate monthly observance of

this temperate species at a single site in the Florida panhandle, exemplifying the persistence of *Ae. japonicus* through all seasons in a subtropical climate.

P-6 Resource acquisition time, shortening diapause periods, and overwintering success of *Culex pipiens* in Illinois

Sara Wilson, smw2@illinois.edu, Christopher Stone

Preparation for overwintering in temperate zones marks a unique ecological and physiological challenge for the West Nile vector *Culex pipiens*. It involves a shift from host-seeking to nectar gluttony in diapausing females to build lipid reserves. An open question is how female mosquitoes should resolve the trade-off between the time needed for resource acquisition and the length of time they need to survive to overwinter. To test this, *Culex pipiens* were reared under diapause-inducing conditions. Upon emergence, females were placed in cages and were provided with water and a honey solution for 8, 4, and 2 weeks, after which sugar sources were removed. A simulated overwintering study using temperature-controlled incubators were performed, with conditions mimicking average winter temperatures in overwintering hibernacula, for the average time between first frost (Fall) to last frost (Spring). This timeline was based on average or projected conditions for Illinois, comparing the years 2000 to 2050. Survival was measured daily. Energetic reserves were measured for a subset of females before and after the simulated winter conditions. I expect to see higher survival rates corresponding to shorter winter periods, and that the optimal period of reserve acquisition will shorten as winters get milder and shorter. Understanding the time needed to build energetic reserves provides insight into the ability of *Cx. pipiens* to adapt to changing winter temperatures and has implications for transmission of West Nile during the fall season.

P-7 Season abundance of *Aedes triseriatus* and other La Crosse virus vectors in endemic areas of Western NC: A comparative approach using ovitraps

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La Crosse encephalitis (LACE) is the most prevalent arboviral cause of encephalitis in children within the United States. In North Carolina, LACE is the most commonly reported arboviral disease in humans. Although known to be regionally endemic throughout many parts of Appalachia, transmission of La Crosse virus (LACV) is focal and risk may persist over time. In 2021, we sampled 13 residential households with a comparative (case vs non-case) study design in an effort to: a) determine seasonal ovitrap abundance of LACV vectors, b) identify transovarial LACV infection rates in western NC, and c) compare infection rates at LACE case and non-case residences. At present, more than 200 residential trap-weeks of collections [>800 trap collections] resulting in >50,000 *Aedes* eggs have been obtained. The seasonal abundance, comparative analyses, and LACV infection rates will be reported.

P-9 Bacterial insecticide changes adult mosquito vector competence for dengue virus
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Dengue virus (DENV) is a worldwide-distributed mosquito-borne virus that affects human health. The use of new naturally derived insecticide for adult mosquito control has potential to reduce the risk of DENV transmission while poses low risk to environment and nontarget organisms. We evaluated the effects of feeding on bacterial insecticide spinosad in sugar solution on adult *Aedes*

albopictus lifespan and vector competence for DENV. Feeding on spinosad heavily lowered the lifespan of adults. DENV infection, dissemination, and transmission rates were higher in spinosad-fed adults than in controls. Loads of DENV in adult tissues were not different between treatments. We show that although feeding on spinosad lowered vector lifespan, it may produce individuals with higher vector competence for DENV. Our results emphasize the need for the assessment of adult control efforts on the epidemiology of mosquito-borne viruses.

P-10 Effect of resistance to pyrethroid insecticides on vector competence and the immune response of *Aedes aegypti* for DENV-2 infection

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Aedes aegypti as the main vector of the dengue virus, has been the main objective of mosquito control programs, however, the development of resistance to insecticides prevents effective control of their populations. Insecticide resistance mechanisms have been reported to affect the interaction between mosquitoes and the pathogens they transmit. In this study we investigated whether resistance to pyrethroids in *Ae. aegypti* has an effect on its vector competence and immune response to DENV-2. Mechanisms based on the overproduction of esterases and MFO enzymes, as well as kdr mutations (Ile 1016 and Cys 1534) were detected in two field mosquito populations from Mexico, Galaxias and Xochimilco, compared to a reference strain susceptible to insecticides, New Orleans. Subsequently, the field mosquitoes and the reference strain were infected with DENV-2. Viral load detection was performed in infected mosquito excreta at 2, 7 and 14 days post infection (dpi), as well as the detection of immune response markers using qPCR at 21 dpi. The statistical analysis indicated that the mosquitoes from Galaxias were more susceptible to viral infection in addition to presenting a lower expression of immune response markers, unlike Xochimilco, which presented a lower susceptibility to infection and a higher expression of immune markers. Our results indicated that these insecticide resistance mechanisms could potentially affect susceptibility to DENV-2 infection. In addition to highlighting the importance of understanding the interaction of resistance mechanisms in the vector competence of *Ae. aegypti* to evaluate the possible consequences in the transmission of disease-causing pathogens in mosquitoes that survive the control methods commonly used in public health.

P-11 Evidence for metabolic resistance in permethrin-resistant *Anopheles gambiae* s.l populations from Delta State, Nigeria.

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Insecticide resistance in malaria vector populations is one of the major challenges confronting malaria control in Nigeria. This challenge can lead to failure of vector control interventions, especially Insecticide treated Nets (ITNs) if not appropriately managed. However, sound management can only be achieved with adequate knowledge of spread and mechanism of resistance. Hence, this study evaluated resistance status and involvement of metabolic resistance in *Anopheles* populations from Delta State to permethrin. Anopheline larvae were collected from three communities and reared till adult at Entomology Laboratory, Delta State University, Abraka. Adult female mosquitoes aged 3-5 days were exposed to 0.75% permethrin using WHO bioassay method. Also, cohort were exposed to PBO and further exposed to permethrin. Exposed mosquitoes were identified molecularly. All identified

mosquitoes were *Anopheles coluzzii*. Populations from Ika-South, Ndokwa-East and Ethiope-West Local Government Area recorded 62%, 74% and 83% mortality respectively. Mortality in synergist assay were 99%, 99% and 100% respectively. Differences in mortality between permethrin and PBO/permethrin-exposed population was significant ($p < 0.0001$). Knockdown Time (KDT50 and KDT95) was lowest in Ethiope-West LGA. Considering increase in mortality value of the populations with exposure to PBO, this study presents evidence for involvement of metabolic resistance in permethrin-resistant *Anopheles* populations in Delta State.

Keywords: *Anopheles gambiae* s.l, Evidence. Metabolic resistance, Permethrin-resistance, Delta State.

P-12 **Mosquito and mosquito borne disease surveillance in the Rio Grande Valley during COVID, 2020-2021**

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In 2017, a joint mosquito surveillance effort between the University of Texas Rio Grande Valley and local city and county public health agencies was initiated through the Western Gulf Center of Excellence in Vector-Borne Disease. In 2020, the introduction and spread of COVID disrupted some of the trapping and testing effort. While incomplete, we present data here for the surveillance results since January 2020. While the trapping effort is still ongoing, to date we processed over 47,000 mosquitoes during that time interval. Mosquitoes are tested for infection using a real-time PCR assay, testing vectors for Zika, Dengue, Chikungunya, and West Nile Virus. As of now, all mosquitoes have tested negative. Mosquito vector species, *Ae. aegypti* and *Cx. quinquefasciatus* are the most prevalent collected female mosquito species, while more uncommon species may occasionally make up a huge proportion of mosquitoes collected. Through our collection data, we discovered that mosquito vector species were present and active throughout the year including winter months when surveillance and control efforts are normally reduced. These data highlight the importance of year-round surveillance in sub-tropical areas where vectors may continually play a continual role in disease transmission.

P-13 **Continued tick and flea surveillance for the detection of tick- and flea-borne disease in the Rio Grande Valley 2019-2021**

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The Rio Grande Valley, located in south Texas, remains a region with limited surveillance efforts and screening for tick- and flea- borne diseases. In 2019, we initiated a project to study tick and flea population and distribution patterns along the Texas-Mexico border and test tick and flea specimens for bacterial pathogens. Our efforts consist of collection, identification, and detection of pathogens. We collaborated with local veterinarian clinics, animal shelters, and the United States Department of Agriculture in the collection of ticks and fleas, as well as conducted field collections using the dragging method, CO2 traps, and flea traps. To date, a total of 2,345 ticks and 398 fleas have been collected. Only four tick species and 5 flea species were collected across the Rio Grande Valley through our collection methods. The most prevalent species for ticks and fleas were *Rhipicephalus sanguineus* consisting of 66% of the collected ticks, and *Xenopsylla cheopis* which made up 47% of the collected fleas. PCR results detected *Rickettsia amblyommatis* in four ticks consisting of two *Amblyomma maculatum*, one *Rh. sanguineus*, and one *Dermacentor variabilis*. Additionally, there were inconclusive results for twelve ticks. Screening and testing for the fleas are pending, as well as 2,084 tick samples. Future plans for the project include the expansion of field collection sites, implementation of more

collection methods, and screening of human serological samples for the detection of Rickettsial agents. The study will highlight the prevalence of tick- and flea-borne disease in the Rio Grande Valley, a region with limited surveillance activity. In addition, our findings will ideally help promote public health control efforts and inform diagnosis by medical professionals.

P-15 Creating an online webinar training series for Florida’s public health pest control licensees

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In Florida, the primary mechanism for licensed public health mosquito control employees to earn continuing education units (CEUs) has been through in-person trainings. These options were unavailable in 2020 due to the ongoing COVID-19 pandemic, leaving many individuals without options to obtain required CEUs. In response, mosquito control specialists working for the Florida Department of Agriculture and Consumer Services developed online webinar classes using funds from the Center for Disease Control and Prevention’s Public Health Crisis Cooperative Agreement Grant for Hurricane Response. Instruction included core requirements, integrated pest management, and hurricane preparedness. Despite not being able to teach in-person, the outreach specialists were able to use several features of the online training platform (GoTo by LogMeIn) to communicate and provide opportunities for participants to ask questions and engage in discussions. The application also allowed the instructors to launch an exam required for Florida CEUs. In addition, the team worked to create a fun, informal online training environment for the attendees. To measure the effectiveness of each training, a survey was deployed through the online application, which resulted in 90% efficacy over a total of 25 trainings. Due to the positive feedback from attendees and the current needs expressed by mosquito control program managers, classes continue to be offered beyond the initial grant period.

P-16 Advancements in GIS capability for Florida’s Mosquito Control Incident Response Team (MCIRT)

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Since the Florida introduction of West Nile virus in 2001, the Mosquito Control Incident Response Team (MCIRT) has been deployed by the Florida Department of Agriculture and Consumer Services (FDACS) to assist impacted counties following an emergency declaration, such as a hurricane or an arbovirus threat. GIS are vital to implementation of all aspects of MCIRT response. This includes conducting mosquito surveillance, maintaining open communication with stakeholders, and contracting applications in order to suppress targeted mosquito populations. Previous GIS capabilities were limited to static maps, delayed updates, and partial data available. With more advanced technology at hand, it is imperative to improve the mapping quality for these incidents by providing dynamic maps with editable features that contain previously unreported data. These changes will provide more proficient GIS that both produce easier to read maps and increase overall efficiency of the MCIRT during response.

P-18 The Comparison of Efficiency Parameters: VectoBac WDG vs. Natular SC

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Collier Mosquito Control District implements an Integrated Mosquito Management approach, utilizing various control materials and application equipment to meet the mission. Up until recently, the District was mostly using VectoBac WDG (*Bacillus thuringiensis israelensis*) water

dispersible granule to control various container-inhabiting mosquito species. In 2021, the District began using the Spinosad-based formulation, Natular SC. Here we report a comparison of efficiency parameters between the two materials, such as, cost, mix processes, acreage covered, target species control, label restrictions and toxicity, and application environments.

P-19

The WALs to the cemetery: Examining efficacy under field conditions

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Vases, plant trays, and other man-made containers placed at gravesites in cemeteries are at high risk for *Aedes aegypti* (L.). In 2019, *Ae. aegypti* were discovered in containers at the Visalia Public Cemetery, with a 5% container index (CI, percent of water-holding containers positive for larvae or pupae). Despite extensive physical control efforts and public education, the cemetery continued to produce *Ae. aegypti* the following year with the CI reaching 40% by the first week of August 2020. This study evaluated the use of truck-mounted Wide Area Larvicide Spray (WALS) to control *Ae. aegypti* under field conditions. VectoBac WDG was applied in the cemetery once a week for four weeks using a truck-mounted A1 Super Duty fogger. Larval abundance was monitored using a randomized container survey. Female *Ae. aegypti* abundance was monitored using BG Sentinel mosquito traps. The CI rapidly decreased from 40.0%, before the first WALS application, to 5.4% by the fifth week with changes in adult *Ae. aegypti* abundance lagging about two weeks behind changes in the CI. While WALS applications reduced *Ae. aegypti* abundance, the ability of mosquito and vector control districts to maintain control of *Ae. aegypti* could be strained by the short effect duration, particularly if multiple areas need to be treated at the same time.

P-20

Evaluation of different larval diets for standardized mass-rearing of *Wolbachia*-infected *Aedes aegypti* for population suppression

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The implementation of *Wolbachia* Incompatible Insect Technique (IIT) method depends on highly efficient mosquito rearing, which depend on standardized larval diets to ensure production of high-quality laboratory-reared mosquitoes. We compared the effect of different larval diets, which vary in their protein source: fish-food for Tilapia (TIL); bovine liver powder (COW); and porcine powder meal (PIG) with a reference diet (MFOOD), on the life history *Wolbachia*-infected (wMID) and non-infected (MID) *Aedes aegypti* under laboratory conditions. The average development time was between 6 and 7 days for wMID and MID mosquitoes, respectively. The pupation rate observed to fall between 92-95% for wMID and 96- 38 98% for MID and no significant differences were observed. The average size for male pupae showed some significant variability depending on the diet. The average time for adult emergence for wMID ranged from 7.90 ± 0.06 to 8.48 ± 0.15 days for males and from 8.27 ± 0.06 to 9.15 ± 0.29 days for females. *Wolbachia* infection was not affected under any of the four larval diets assessed. All four diets were comparably suitable to rear and maintain colonies of *Ae. aegypti*, regardless of the *Wolbachia* infection status. No negative impact was detected on the developmental cycle for both wMID and MID and based on cost, the local availability of ingredients and published results, the diets PIG and TIL are good and recommendable options. These two low-cost and suitable

diets could be used for *Ae. aegypti* mass-rearing systems (both *Wolbachia*-infected and non-infected) in Mexico and, potentially, as food for other *Aedes* species, such as *Ae. albopictus*.

P-21 **Delta Mosquito and Vector Control District's wireless smart mosquitofish feeder system**

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With the completion of the Alburn Fish Hatchery, i.e., indoor mosquitofish rearing facility in October 2020, Delta Mosquito and Vector Control District (DMVCD) aims to optimize mosquitofish breeding, growth, and development by adding innovative features and equipment to the facility. Challenges faced in the aquaculture industry around feeding include time, maintenance, programmability, modifications, user interfaces, and budgetary constraints surrounding fish feeders and compatible controllers. With the realization that nothing on the market fits the needs and desires of the District's mosquitofish program, DMVCD designed and developed a novel automatic feeding system using a DC-powered lawn sprinkler controller, wireless RF transmitters, and Pentair AES 3L Vibratory Feeders. This cost-effective alternative features an open-sourced Wi-Fi smart controller that allows programming through a web browser or mobile app, scheduled automatic feeding programs for each feeder, control of up to 71 unique feeders or other electronic devices, and wireless RF transmitters that allow the device to send signals up to 55 meters away from the unit. This presentation highlights the design and building process of the automatic feeding system and its success as a cost-effective solution that can be adopted by any small to mid-sized aquaculture system.

P-22 **Elimination of *Aedes aegypti* mosquitoes from Culebra, Puerto Rico**

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The *Aedes aegypti* mosquito is the primary vector of dengue, Zika, and chikungunya viruses in Puerto Rico. This mosquito is present year-round on the Island, as is the public health threat from these endemic arboviruses. In 2019, The Puerto Rico Vector Control Unit (PRVCU) and the Rotary Club of San Juan implemented a community assessment with crucial stakeholders showing Culebra municipality has sufficient strong community support to attempt to eliminate *Ae. aegypti* from the island. Thus, we began a project to sustainably eliminate or reduce to negligible levels the *Ae. aegypti* mosquito populations on Culebra over two years. Culebra is a small island (10 square miles, 1,500 inhabitants) in the U.S. Territory of Puerto Rico and is ideal for conducting such a project. However, the local authorities have no vector control plan, no mosquito surveillance, and no ongoing community education about *Ae. aegypti*. We used AGO traps as our primary surveillance tool and established that the initial population was 6.55 females/trap/week (ranging from 0 to 96). The PRVCU also conducted yard inspections and established that over 95% of the yards had breeding sites. Each yard contained an average of about 4 containers with water, about half of which were positive for *Ae. aegypti* larvae. A Knowledge, Attitudes, and Practices (KAP) survey was used to assess citizen compliance with recommendations showing that our initial outreach effort was at least 50% successful. This presentation will provide additional information to be obtained over the next 7 months.

P-23 **Evaluation of emergence trap design for the assessment of *Mansonia* production from habitat containing water lettuce (*Pistia stratiotes* L.)**

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Larvae of the mosquito species *Mansonia titillans* (Walker) and *Mansonia dyari* (Belkin, Heinemann & Page) attach to the roots of floating aquatic plants, primarily water lettuce (*Pistia stratiotes* L.) and water hyacinth (*Eichhornia crassipes* (Mart) Solms), to obtain oxygen and avoid predators. Surveillance for these species involves a robust monitoring program that identifies *Mansonia* habitat and production sites. Control measures include reduction of the host plants, as well as targeting the immature and adult stages of the mosquitoes. This report evaluates floating emergence trap efficiency for *Mansonia* surveillance and identification of production sites. Three trap designs were utilized in the evaluation trials, including standard passive emergence traps, modified (active) emergence traps containing a CDC-light trap and modified (active) emergence traps containing a CDC-light trap with no light.

P-24 **Quality control of *Wolbachia*-infected *Aedes aegypti* eggs under a mass-rearing system**

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Aedes aegypti is considered the most important vector of human diseases (dengue, chikungunya and Zika) and the main vector of dengue in Mexico. Promoted by WHO, the integrated vector management is the main method for tackling many of the world's most burdensome infectious diseases such as dengue and other neglected tropical diseases. Innovative biological control methods have been used in some countries to control *Aedes* spp., such as the sterile insect technique (SIT) and the incompatible insect technique (IIT), as standalone approaches or used in combination. IIT is based on mass-rearing of the target species and continuous release of the sterile insects into the target population; however, there are many aspects requiring special attention to move to the operational level. The shelf-life and management of eggs is an important step in quality control for mass release programs because typically eggs are produced, stored and transported to sites prior to release or rearing for further production. Here, we presented results on fecundity and fertility of female *Ae. aegypti* carrying *Wolbachia* (wAlbB) under a blood-feeding scheme. Additionally, viability of *Wolbachia*-infected eggs are presented according to adult mosquito age and viability over time under laboratory conditions.

P-25 **Quality control of sterile *Aedes aegypti* mosquitoes used prior field release in a combined SIT-IIT approach**

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Sterile insect technique (SIT) and the incompatible insect technique (IIT) are now available innovative biological approaches to control wild mosquito populations worldwide, particularly in endemic areas where arboviral diseases such as dengue, Zika, and chikungunya are highly prevalent. Both techniques require the release of large numbers of sterile males, which it needs of a high-quality control of the mass-reared mosquitoes. The male mass production for release includes five laboratory quality control procedures female contamination rate by terminalia examination, pupal size measuring the width of cephalothorax in their ventral view, assessing of *Wolbachia* infection, cytoplasmic

incompatibility (CI) and male competitiveness (C). We showed that female contamination rate during mass-rearing varied between 0.02-0.02%, the average of pupae sizes range between 1.00 mm for males and 1.21 mm for females, Wolbachia infection was maintained through two distinct mosquito generations (F29, F30) being fixed (100%) in all individual tested, the CI was 100% and the C was 1.29 with a residual fertility of 0.43 and induced sterility (IS) of 99.57%. The experiments included in this work are the basis of laborious and strictly laboratory quality control procedures to ensure high quality of the sterile mass-produced mosquitoes in IIT-SIT programs. Our quality control procedures in the mass-production of mosquitoes reduces the probability of releasing fertile females which prevented the risk of population re-placement. Additionally, all male mosquitoes resulted 100% incompatibles and fully competitive at the irradiation doses used.

P-26 Detección de Aedes (Stegomyia) albopictus (Skuse) en ovitrampas en Mérida, México
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El programa de enfermedades transmitidas por vectores en México tiene una red establecida de ovitrampas para la vigilancia entomológica de Aedes spp. Los servicios de salud del estado de Yucatán, en respuesta a reportes de Aedes albopictus en la periferia de Mérida, capital del estado, incrementaron la especificidad de dicha vigilancia. El objetivo fue describir la presencia y distribución de Ae. albopictus en Mérida y su abundancia relativa comparada con Aedes aegypti, en ovitrampas del programa de control de vectores. Durante octubre de 2019, se seleccionaron al azar 91 ovitrampas en 31 barrios de Mérida. Los mosquitos adultos se obtuvieron del insectario de la Unidad Colaborativa para Bioensayos Entomológicos de la Universidad Autónoma de Yucatán a partir de huevos recolectados en campo. Se determinó la abundancia relativa de individuos adultos de cada especie identificada y por barrios evaluados. En el 32 % de los barrios muestreados, se detectó Ae. albopictus y, en todos ellos, Ae. aegypti. Se recolectaron 28 adultos de Ae. albopictus (10 hembras y 18 machos) en las ovitrampas. No se observó correlación entre la abundancia de adultos ni de hembras Ae. aegypti y Ae. albopictus por barrio ($p>0,05$). Los resultados confirmaron que Ae. albopictus estaba coexistiendo con Ae. aegypti en Mérida en el momento del estudio. La baja abundancia relativa sugiere que Ae. albopictus se encontraba en la fase inicial de invasión.

P-27 Evaluation of the susceptibility to organophosphates in Culex quinquefasciatus from the department of Atlantico, Colombia
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The status of susceptibility to organophosphates was evaluated in two populations of Culex quinquefasciatus from the department of Atlantico, Colombia. Bioassays for temephos, malathion and pirimiphos-methyl were carried out in third instar larvae and adults of Cx. quinquefasciatus collected from the municipalities of Soledad and Puerto Colombia. The methodology of the WHO (1981) and the CDC (1998) were used. The resistance ratio (RR) of the LC50 was calculated for each insecticide in the evaluated field populations. The susceptible strain Cartagena was used as a reference control. The populations of Cx. quinquefasciatus were susceptible to all the organophosphates evaluated

(RRCL50<5.0) except for malathion in the population of Puerto Colombia in which moderate resistance was found (RRCL50 8.6). Taking into account the results obtained, the use of organophosphates is recommended for the control of *Cx. quinquefasciatus* in the two populations evaluated with exception of malathion in *Cx. quinquefasciatus* from Puerto Colombia.

P-28 New geographic distribution records of *Clogmia albipunctata* Williston 1893 Diptera Psychodidae in Venezuela

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Clogmia albipunctata (Williston, 1893), is a fly of the family Psychodidae, the adults are found in humid environments inside and outside house; its larval stages live in wetted unclean areas as bathrooms, toilets and sewage. It causes different forms of myiasis as nasopharyngeal, intestinal, and urinary myiasis and are considered as potential mechanical transporters of pathogenic microorganisms in unsanitary environments. Between January 2017 and April 2020 in daylight hours the adult specimens in different areas of Venezuela were collected directly from their resting places using glass aspirators and/or observed and photographed in situ. Comparative taxonomic morphological identification was carried out following the descriptions and taxonomic keys of Forattini (1973) and Ibañez-Bernal (2008). The results obtained allow us to report the presence of drain bathroom fly *C. albipunctata* in eleven localities of Venezuela: Central region: Distrito capital, Caracas (El Paraíso [10°29'11,61"N, 66°55'45,40"O]); Sistema Coriano: Estado Falcón (Coro [11°56'25"N, 69°53'7"O]); Punto Fijo [11°43'00"N, 70°11'00"O], La Peña [1°6'29"N, 69°44'29"O] and Maparari [10°47'41"N, 69°26'36"O]) y Estado Lara (Barquisimeto [10°04'04"N, 69°20'48"O]). Región oriental: Estado Anzoátegui: (San José de Guanipa [08°53'00"N, 64°09'00"O] y Anaco [09°26'00", 64°28'00"O]). Región de Los Andes: Estado Trujillo (Trujillo [09°22'00"N, 70°25'59"O]) y Estado Mérida (Mérida [08°33'32,8"N, 71°11'58"O]). Región de los llanos: Estado Apure (Guasdalito [07°14'48"N, 70°43'45"O]).

Key Word: Distribution, drain bathroom fly, taxonomy, Venezuela.

P-29 Susceptibility to pyrethroids and kdr mutation L1014F in populations of *Culex quinquefasciatus* in Colombia

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We evaluated the status of susceptibility to pyrethroids in two populations of *Culex quinquefasciatus* in the department of Atlántico (Colombia) and its possible mechanism of resistance (kdr mutation).

Bioassays were performed for permethrin, deltamethrin, and λ -cyhalothrin in *Cx. quinquefasciatus*, according to the CDC methodology. The resistance ratios were determined to KC50 and LC50 for each insecticide in the field populations and the susceptible strain Cartagena. The kdr mutation L1014F was identified in the para gene for the voltage-dependent sodium channel and its allelic and genotypic frequency.

Moderate knock-down resistance to permethrin was observed in the populations of Puerto Colombia (RRKC50: 5x) and Soledad (RRKC50:7x), low resistance to deltamethrin in both populations (RRKC50: 4,6x y 1,9x), while for λ -cyhalothrin moderate resistance was observed in Puerto Colombia (RRKC50: 5x) and low in Soledad (RRKC50: 3,8x). In relation to permethrin, low resistance at 24 hours post-exposure

was observed in *Cx. quinquefasciatus* from Puerto Colombia (RRLC50: 3,9x) and moderate in Soledad (RRLC50: 7,6x); moderate resistance for deltamethrin in Puerto Colombia (RRLC50: 5,1x) and low in Soledad (RRLC50: 4,5x); in relation to λ -cyhalothrin, low resistance was observed in Puerto Colombia (RRLC50: 3,6x) and moderate resistance in Soledad (RRLC50: 5,2x).

With respect to the L1014F mutation, allelic frequencies of 0.28 were determined for *Cx. quinquefasciatus* from Puerto Colombia and 0.38 for Soledad.

Variability in the status of susceptibility in the populations of *Cx. quinquefasciatus* evaluated was found. We also report for the first time for Colombia the *kdr* mutation L1014F as a possible mechanism of resistance to pyrethroid in this species.

P-30 Susceptibility to organophosphates in *Aedes aegypti* from the department of La Guajira, Colombia

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The status of susceptibility to organophosphates was evaluated in ten populations of *Aedes aegypti* in the department of La Guajira (Colombia). Bioassays were performed for temephos, malathion, and pirimiphos-methyl in the third instar larvae and adults of *Ae. aegypti* from the municipalities of Albania, Dibulla, Riohacha, Manaure, Barrancas, Uribia, Fonseca, San Juan del Cesar, Villanueva, and Maicao following the methodology of WHO (1981) and the CDC (1998). Susceptibility was determined through the resistance ratio (RR) of LC50 for temephos and the frequency of resistance using the diagnostic dose (DD) for temephos, malathion, and pirimiphos-methyl in the evaluated field populations. The Rockefeller strain was used as a susceptible control. The populations of *Ae. aegypti* were susceptible to temephos (RRLC50<5.0; 98% - 100% mortality); pirimiphos-methyl (99% -100% mortality) and malathion (100% mortality). Taking into account the results obtained, the use of temephos, malathion, and pirimiphos-methyl is recommended for the control of *Ae. aegypti* in the populations from La Guajira, Colombia.

129 Seasonal host selection in Rio Grande Valley WNV vectors

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West Nile Virus (WNV) is the most geographically well-distributed arthropod virus in the world, in no small part due to the mobility and habitat diversity of avian species, WNV's key reservoir hosts. In spite of this, how arthropod vectors of WNV interact with migratory birds is not fully understood in all areas of epidemiological importance. One of these areas of oversight lies in the Rio Grande Valley (RGV) area of South Texas, which boasts both an important avian migration route, and high incidences of WNV transmission.

In order to shed light on the predation dynamics between South Texas WNV vectors and hosts, blood-fed mosquitoes were collected in natural areas commonly visited by migrating birds. Vectors were trapped during seasons of both low and high avian migration to ascertain how vector feeding preferences fluctuate in response to changes in host availability. Genetic material extracted from the mosquito blood-meals was subjected to DNA barcoding procedures in order to identify the host species of these vectors. Future findings derived from these genetic assays will be discussed during the

presentation. Data obtained from this research will deepen our understanding of WNV transmission cycles and enable medical entomologists to more clearly discern the risks of WNV emergence in the RGV.

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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)		
1983	Harry D. Pratt (GA)	2004	James D. Long (TX)		
	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)	1996	Carl R. Tanner (IL)	2010	Gordon Patterson (FL)
	Leslie E. Fronk (UT)		Sammie L. Dickson (UT)		Gary Clark (FL)
	Jesse B. Leslie (NJ)	1997	Charles T. Palmisano (LA)		Yasmin Rubio-Palis (Venezuela)
1981	Linda G. Raiche (CA)		George J. Wichterman (FL)	2011	Angela Beehler (WA)
	Margaret S. Slater (NY)	1998	Douglas B. Carlson (FL)		Roxanne Connelly (FL)
1982	K. G. Nolan (NY)	1999	Charles Beesley (CA)	2012	Truc Dever (CA)
	Charles F. Scheel (IL)		Donald R. Johnson (GA)	2013	Robert Peterson (MT)
1983	Coyle E. Knowles (NY)	2000	Peter B. Ghormley (CA)	2014	Salvador Rico (TX)
1984	Ray Treichler (DC)		David A. Brown (CA)	2015	Kristy Burkhalter (CO)
1985	Lawrence T. Cowper	2001	Donald Menard (LA)		Elizabeth Cline (CA)
	Janice B. Wells (NY)		Joel Margalit (Israel)	2016	Angela Beehler (WA)
1986	T. Oscar Fultz (GA)	2002	Dennis Moore (FL)		John Biedler
1987	Sharon A. Colvin (IL)		Henry R. Rupp (NJ)	2017	Peter Connelly (FL)
1988	Daniel D. Sprenger (TX)	200	James R. McNelly (NJ)		Larry Smith (GA)
1989	Fred C. Roberts (CA)		Robert Bonnett (MN)	2018	Stephen Sickerman (FL)
1990	Leonard E. Munsterman (IN)	2004	James R. Brown (FL)		Isik Unlu (NJ)
1991	James D. Long (TX)	2005	Mark Newberg (IL)	2019	Brian Byrd
1992	Charlie D. Morris (FL)		Susan Maggy (CA)		Rui-de Xue
1993	Robert J. Novak (IL)	2006	Teung Chin	2020	Levy Sun (CA)
1994	James W. Robinson (FL)	2007	Karl Malamud-Roam (CA)		Harry Savage (CO)
	Dan L. Ariaz (NV)	2008	William H. Meredeth (DE)	2021	Gary Hatch (CA)
1995	Sally Kuzenski (LA)	2009	Rep. Dennis Cardoza (CA)		Kristen Healy (LA)
				2022	Catalina Alfonso-Parra (Columbia)
					Tianyun Steven Su (CA)

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 Rattanarithkul, 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)
 1998 Ralph E. Harbach (UK)
 1999 Yiau-Min Huang (DC)
 2000 Lewis T. Nielsen (UT)

2020 Jan Conn
 2021 Ken Linthicum
 2022 Chet Moore (CO)

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. Gray	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 Anderson B. Ritter	C. Lamar Meek	Les maringouins du mech: The legacy of two men
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. Knippling	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

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

GRASSROOTS AWARD

This award is given to recognize excellent performance and dedication by mosquito control field staff.

2005	Omar S. Akbari	Reno Washoe County, 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
	Geneva Ginn	Coachella Valley MVCD, California
2015	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 Dewsnup	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

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
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	2018	Annie Rich***	University of Georgia

STUDENT PAPER COMPETITION AWARDS

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	2019 Casey Parker*	
	Rebecca Trout*	University of Kentucky	Ed Norris**	
2006	Robert D. Anderson	University of	Meredith Beaulieu***	
	Linda O'Connor**	University of	Raji Joshua***	
	Joshua R. Ogawa*	Oregon State	Christopher Bibbs***	
	Matthew Eaton*	Concordia College	2021 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		
	Christopher Barker*	U. California, Davis		
	Lisa Reimer*	U. California, Davis		

* \$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

* - Eastern Association of Mosquito Control Workers

AMCA TREASURERS

1935-1943	Thomas D. Mulhern *	1994-2000	Charles T. Palmisano
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		

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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

1995-1999	Pamela D. Toups
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