

Submitted papers,  
posters, and symposium  
presentations



# AMCA 2014

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**The 2014 AMCA Memorial Lecture Honoree:  
Founding Mothers: Women, Entomology and the Origins of the  
Mosquito Control Movement**

The role women played in the development of entomology in general and mosquito control in particular is a story that reveals courage, intelligence, and dedication. From European entomologists like Eleanor Glanville and Maria Sibylla Merian in the 17<sup>th</sup> and 18<sup>th</sup> centuries to Americans such as Mary Treat, Anna Comstock, and Evelyn Mitchell in the 19<sup>th</sup> and early 20<sup>th</sup> centuries, women played a critical role in advancing our understanding of the insect world. The pioneering work of these women and others has been far too long untold.

This Memorial Lecture will be dedicated to five unsung heroines who contributed mightily to raising the mosquito control movement.

Carrie B. Aaron (1858-1954) Winner in 1890 of the Lamborn Prize given in competition for most outstanding essay on mosquito control

Clara S. Ludlow (1852-1924) Taxonomist, Lecturer in Medical Entomology Army Medical Museum

Vida L. MacDonell (1886-1969) Civic Leader and Activist, First Woman President of Florida Mosquito Control Association

Helene Trembley Durkee (1905-1983) Entomologist, Associate Editor of *Mosquito News* (1948-1969), Author *AMCA Bulletin 3 Mosquito Culture Techniques and Experimental Procedures* (1955)

Elizabeth Basham Thurman-Swartzwelder (1920-1987) Scientist and Public Health Worker (Malaria Control in War Areas, United States Public Health Service and National Institutes of Health)

\* Thurman-Swartzwelder, Ernestine. "Needed: more women in science." *Nursing outlook* 9 (1961): 634.

## The 2014 AMCA Memorial Lecturer: Gordon Patterson



Gordon Patterson is a professor of history at Florida Tech, where he has taught since 1981. He was a lecturer at the University of

Maryland from 1974 until 1981, and an instructor at UCLA in 1973. He holds two graduate-level degrees from UCLA, as well as a B.S. from Northwestern University.

Before coming to Florida Tech, Gordon spent eight years in Europe working in Vienna and Heidelberg. He held a Fulbright Fellowship at the University of Heidelberg. He has received six National Endowment for the Humanities awards to attend NEH Seminars and Institutes. In 1997, 1999, and 2001 Gordon was invited to the East-West Center at the University of Hawaii. Gordon has served as a visiting professor in both Korea and Japan. In 2004, he received a Fulbright Travel Award for work in Malaysia and Indonesia. In the summer of 2011, Gordon and his wife, Joy, were invited by the Chinese Ministry of Education to visit Chinese universities in Beijing, Shanghai, and northeast China.

At Florida Tech, Gordon has received the faculty excellence award for service in 1988-1989; the faculty excellence award for teaching in 1992-1993; the Sustained Commitment to Student Life Award in both 1995 and 1997; the Florida Tech Student Alumni Award for Outstanding Teaching in

1995; and in October, 1996 he was selected the first winner of the MVP (Most Valuable Panther) award. In February 1997, Patterson received the Lambda Chi Alpha fraternity Outstanding Faculty Award. In 1999, he received the Alpha Phi Foundation Chapter Award for Excellence in Teaching. In 2011, Gordon was named the first recipient of the Florida Tech Alumni Award for Lifetime Service to the university.

Gordon has served as the Social Science Chair of the Florida Academy of Sciences, a director of the Florida Humanities Council; and a director of the Florida Historical Society. He has written numerous articles which have appeared in both scholarly and popular publications. In 2004, the University of Florida published his *The Mosquito Wars: A History of Mosquito Control in Florida*. Five years later, Rutgers University Press released *The Mosquito Crusades: A History of the American Anti-Mosquito Movement*. In 2004, Gordon received the Florida Mosquito Control Association's Presidential Award for his work in advancing the cause of mosquito control in Florida. In 2010, the AMCA recognized Gordon's contributions to the mosquito control community by naming him recipient of the association's Presidential Award.

Gordon is married to Joy Patterson who is his colleague at Florida Tech. A life-long Episcopalian, he has served as a member of Holy Trinity Episcopal Day School's School Board as well as the board's chair. Currently, Gordon is a member of the board of directors of the Brevard Neighborhood Development Coalition.

## Oral Presentation Abstracts

### Plenary Session

#### PL-1 Accelerating to zero: strategies for achieving malaria eradication

Alan J. Magill, alan.magill@gatesfoundation.org

Over the past decade, current malaria control interventions and strategies applied at large scale have reduced global malaria mortality by 25 percent. While this progress is unprecedented, malaria still kills nearly 700,000 people and results in more than 200 million cases every year. While these interventions and strategies remain effective and save lives in most settings, they entail high long-term costs and are threatened by drug and insecticide resistance. New interventions and new strategies are needed for accelerating the pathway to eradication and the best way to sustain the progress gained by high level control and to prevent resurgence. Investments leading to new insights and innovations in the science of eradication and flexible delivery models can help speed the trajectory to malaria eradication by detecting and eliminating the human reservoir of infection in asymptomatic persons combined with effective and complete transmission prevention. While sustaining current gains is imperative, a new emphasis on achieving the goal of eradication is vital today.

#### PL-2 Arbovirology: paying it forward

Charles H. Calisher, calisher@cybersafe.net

Since the middle of the last century, the term “arbovirology” has been used to describe the study of viruses transmitted by hematophagous arthropods. Even more than 50 years before that, investigators studying viruses causing yellow fever, African horse sickness, Nairobi sheep disease, and many other medically-important illnesses, made significant and fundamental contributions to our knowledge of and competence in disease description, epidemiology, diagnosis, even viral taxonomy. The arboviruses are not a taxon or class of viruses but are grouped together because of epidemiologic commonalities. Now, as remarkably sensitive molecular techniques are being employed and adapted, it is well for us to look back and recognize the influences of these initial findings and how they have spread from narrow studies to virology as a whole. This presentation will attempt to demonstrate some of the roots of virologic findings and show where they led to vaccine development, rapid diagnosis, and our understanding of the role of vectors in natural cycles of diseases (and non-diseases).

### Adult Control

#### 1 Aerial spraying Anvil 10+10® in the high desert (Casper, Wyoming) for *Aedes dorsalis* and *Culex tarsalis*

John Leman, johnle@cncd.org, Fran Krenick and Clark Wood

This paper will discuss the challenges of aerial spraying for *Aedes dorsalis* and *Culex tarsalis* in Natrona County (Casper), WY. Timing is essential for ULV adulticide sprays to target actively flying mosquitoes and has proven to be even more critical for aerial applications in Casper. In general, most mosquito species targeted by ULV fly in the crepuscular hours, and, hence, most adulticide applications occur in the crepuscular hours. It has been reported in northern Colorado (Godsey et. al., 2010) that peak host-seeking activity for both species is highest between sunset and 6 h after sunset, however this does not appear to be the case in Natrona County, WY. *Aedes dorsalis* and *Culex tarsalis* activity period in Natrona is supported by observations and trapping data which will be presented in this paper. This area of Wyoming is also influenced by mountain meteorology and often the conditions during mosquito activity periods are not conducive to aerial ULV applications.

#### 2 Oviposition deterring and oviciding potentials of *Ipomoea cairica* leaf extract against dengue vectors

Wan Fatma Zuharah, wfatma@usm.my, Rattanam AhbiRami, Maniam Thiagaletchumi, Nik Fadzly, Hamady Dieng, Abu Hassan Ahmad and Sazaly AbuBakar

Because of the drawbacks of chemical insecticides, phytochemicals of plant origin with mosquito control potential are being utilized as alternative sources in integrated vector control. The present study deals with laboratory experiments to determine oviposition deterrent and ovicidal activity of crude extract obtained from herbaceous perennial plant, *Ipomoea cairica* Linn. (Family: Convolvulaceae). Oviposition deterrent activity and ovicidal assay was carried out in oviposition site choice experiments with 3 different concentrations (50, 100, 450 ppm) and control contained only seasoned water. Acetonilic extract of *I. cairica* leaf strongly inhibited oviposition with 100% effective repellency by *Aedes aegypti* at the lower concentration of 100 ppm, while for *Ae. albopictus* was at 450 ppm. The oviposition activity index (OAI) values, which ranged from -0.69 to -1.00, revealed that *I. cairica* had a demonstrated deterrent effect. In an ovicidal assay, a similar trend was observed whereby zero hatchability was recorded for *Ae. aegypti* eggs at 100 and 450 ppm for *Ae. albopictus*. It is noteworthy that *I. cairica* leaf extract had significantly elicited dual properties as oviposition deterrent and oviciding agent in both *Aedes* species at the given concentrations. Reduction in egg number through oviposition deterring activity and reduction in hatching percentage suggested an additional reason for this plant to be integrated in *Aedes* mosquito control as new novel plant based insecticides in future.

**3 In vitro bioassay of a new Florida "green" product registered for adult mosquito control**

John P. Smith, jsmith@pc.fsu.edu

A proof of concept study was performed to assess the effect of a proprietary botanical mixture known as EverSafe™ on laboratory-reared *Aedes aegypti*. Replicated laboratory trials employing the K&D repellent testing system were conducted to measure feeding deterrence and determine the time post-exposure when this behavior was maximized. Additional trials were conducted to substantiate feeding deterrence at the peak exposure period. Ambient environmental data were collected as well. Sufficient evidence was compiled to support advancing this product to a field testing phase.

**4 Sand fly control in Kenya with a backyard misting system**

Seth C. Britch, seth.britch@ars.usda.gov, Kenneth J. Linthicum, Robert L. Aldridge, Thomas M. Logan, Joshua D. Bast, Elizabeth Wanjia, Vitalice O. Opondo, Daniel N'Gonga and Cliff K. Chepchieng

US military operations in hot-arid regions face significant impacts from disease-vector and nuisance populations of filth flies, mosquitoes, and sand flies. Through the Deployed War Fighter Protection Program (DWFP), we have developed a number of techniques to reduce populations of these insects or to reduce insect-human contact in desert areas, including the application of residual pesticides to materials commonly found in military settings such as HESCO blast walls. In this study we investigated the extent that a commercially produced back yard pesticide misting system could be synergistically arranged with HESCO units that were pre-treated with the residual pesticide  $\lambda$ -cyhalothrin in a hot-arid region in western Kenya targeting local abundant populations of *Phlebotomus* sand fly vectors of *Leishmania*. In misting trials using both botanical and permethrin formulations, sand fly numbers in CO<sub>2</sub>-baited CDC light traps were substantially reduced at treated and untreated HESCO units with misters compared to treated HESCO units without misters and an untreated control HESCO without misters. We discuss the relative value of misting with botanical formulations, which may repel sand flies, compared to permethrin formulations, which are expected to produce mortality.

**5 Development of a smart mosquito counter: Can we measure mosquito population?**

Hoonbok Yi, yih@swu.ac.kr, Jae-seung Yu, Kyungoh Park, Sangae Kim, Wook Gyo Lee, Ehyun Shin and Miyun Park

We are developing the smart mosquito counter (h:1080mm × w: 560mm × diameter 320mm), which can attract female mosquitoes by emitting CO<sub>2</sub> gas (300ml/min), can count the number of the captured mosquitoes by an infra-red beam area sensor, and can send the captured mosquitoes numbers through the CDMA module in real time. We operated the 8-16 devices with mosquito sensor networks and a server at the Youngdeungpo-gu areas in Seoul, South Korea for three years (2011-2013) and we could efficiently control mosquitoes at the high mosquito density area based on the mosquito sensor's data. We found that the accuracy of the device was about over 93% compared the real mosquito data and transmitted data by CDMA. We also found the water reservoir areas to control floods in Seoul had relatively higher mosquito density than other normal areas, because those flat-water areas were preferred by mosquitoes. Because our pest control office knew the mosquito occurrence peak times and areas from mosquito data transmitted by the mosquito sensor devices, we efficiently controlled mosquito larva and adults at the right time. We could accomplish a very systematic mosquito control policy and we had high credibility with the results. Based on the mosquito occurrence data, we selectively and scientifically controlled mosquitoes. Therefore, we reduced pesticide usage and saved annually pesticide expenses up to 2/3. Conclusively, if there are no mosquitoes, we don't have to control mosquitoes.

**6 A *Wolbachia*-based autocidal approach to control *Aedes albopictus***

James W. Mains, jmains@mosquitomate.com, Corey Brelsfoard, Robert Rose and Stephen L. Dobson

The Asian tiger mosquito (*Aedes albopictus*) is an invasive species and public health concern because of its transmission of medically important pathogens (e.g., dengue, chikungunya) and aggressive day-biting behavior. Despite intensive use of insecticides to manage this species, it has colonized much of the US, and in recent years, its range has expanded to include California and New York. A proposed autocidal approach for its control is based on *Wolbachia*, an endosymbiotic bacteria that is common in many insect species. Similar to sterile insect technique, the *Wolbachia* approach is based on the release of *Wolbachia* infected males, which cause a form of conditional sterility in the targeted populations. In July 2013, MosquitoMate Inc. was awarded an EPA Experimental Use Permit (No. 89668-EUP-1) to conduct field performance tests of a *Wolbachia* approach against the Asian tiger mosquito in the continental US. This presentation will summarize early work, examining for *Wolbachia* infected male *Ae. albopictus* performance under field conditions, including tests of longevity and dispersal of released males using mark release recapture (MRR) experiments in infested neighborhoods of Lexington, KY. This report will also briefly summarize plans for future work in Kentucky and additional states.

**7 Merus™ 2.0 - ground ULV application results with a new OMRI Listed organic pyrethrum formulation**

Jason Bakken, jasonwbakken@gmail.com and Jacob Hartle

Merus 2.0 is the first OMRI-listed public health pyrethrum formulation. This paper will discuss the ULV cage trial results utilizing 0.76 oz/acre of OMRI against *Aedes melanimon*.

**8 Mini-Bidlies: Pasco County Mosquito Control District's continued use of Bidlingmayer suction traps for adult mosquito surveillance**

Aaron Lloyd, [alloyd@pascomosquito.org](mailto:alloyd@pascomosquito.org)

In 1986, Pasco County Mosquito Control District (PCMCD) deployed 42 suction traps (ST) across the county for adult mosquito surveillance. Today, PCMCD continues to rely on its ST data to help direct adult mosquito control efforts, while most mosquito control districts rely on CDC and NJ light traps. PCMCD has conducted 2 studies to determine if trap color modifications or the addition of lures will add value to the current adult mosquito surveillance program. A brief comparison of traps used in mosquito control and the results from these studies will be discussed.

## Legislative and Regulatory I

**9 An overview of the AMCA legislative and regulatory issues**

Edward Ruckert, [eruckert@mwe.com](mailto:eruckert@mwe.com)

Mr. Ruckert will present an overview of legislative and regulatory issues affecting AMCA members. Topics will include NPDES permitting changes, federal lawsuits that have an impact on mosquito control, and working with the current Congress.

**10 Mosquito control product registrations**

Karl Malamud-Roam, [kmr@aesop.rutgers.edu](mailto:kmr@aesop.rutgers.edu)

**11 Endangered Species Act considerations**

Michael Hudon, [m.hudon@irmosquito2.org](mailto:m.hudon@irmosquito2.org)

As their habitats are diminished or destroyed by unabated human expansion and as pressure from competing exotic invasive species increases, more and more organisms meet criteria for protection under the Endangered Species Act. This broadening of the regulatory umbrella results in mosquito control efforts being modified, restricted, or eliminated. This presentation will update you on recent ESA related decisions that impact mosquito control.

## Student Competition I

**12 The 25th Student Paper Competition of the American Mosquito Control Association**

Brian Byrd, [bdbyrd@wcu.edu](mailto:bdbyrd@wcu.edu)

Since 1989 the American Mosquito Control Association has encouraged student participation by providing student members a forum to present their research. This venue is judged competitively and winners receive the Hollandsworth Prize, which honors AMCA member Gerald Hollandsworth. Each participating student will have 15 minutes to present their paper.

**13 Malaria transmission risk profiles of Northern South America (NSA)**

Temitope O. Alimi, [t.alimi@umiami.edu](mailto:t.alimi@umiami.edu)

Malaria control and elimination is high on the global health agenda. Since evidence-based targeting is essential for control, risk profiles of areas are required. We examined risk distribution in NSA by producing maps identifying areas of risk for malaria. Data layers of environmental and human factors were used in a multi-criteria evaluation model to map risk. Maps were validated using data on three mosquito species. Risk scores for mosquito points were significantly different from random points.

**14 Effect of La Crosse virus infection on neurotransmitter levels and host-seeking behavior of vector mosquitoes**

Fan Yang, [yangfan@vt.edu](mailto:yangfan@vt.edu)

Previous work showed La Crosse virus infected *Aedes triseriatus* and *Ae. albopictus* take significantly less blood than uninfected mosquitoes and tend to refeed more. Using HPCL-ECD, I showed that the levels of neurotransmitters in the brains of infected mosquitoes are altered compared to controls. Olfactometer experiments were conducted to determine the effect of virus infection host-seeking behavior. Virus induced manipulation of blood-feeding behaviors would facilitate pathogen transmission.

**15 The effect of permethrin based pesticides and their residues on honey bee mortality**

Benjamin Sperry, [sperry.ben@gmail.com](mailto:sperry.ben@gmail.com)

Colony collapse is a concern for many beekeepers, and mosquito control programs are often implicated because of the pesticides used in mosquito control. We evaluated the direct and residual effects of pesticides on bees. Bee hives were exposed to pesticide sprays from truck mounted ULV sprayers and data were gathered on bee mortality due to direct and indirect exposure. Our results indicated direct exposure was lethal but there was no mortality due to indirect exposure or pesticide residues.



### 16 Voltage-sensitive chloride channels as a screen for new mosquito toxicants

Lacey Jensen, ljensen84@gmail.com

Mosquitoes are vectors for a large number of human diseases and kill millions of people a year worldwide. Previous studies have shown that voltage-sensitive chloride channels may represent a novel insecticidal class. We characterized the chloride channels expressed in *Anopheles gambiae* Sua1B cells and identified a unique class of calcium-activated chloride channels. The present experiments screened Sua1B insect cells with chloride channel blockers as a model screening system.

### 17 Development of novel mosquitocides targeting potassium channels for control of mosquito-borne diseases

Nicholas R. Larson, nick.larson@ufl.edu

Development of insecticide resistance continues to threaten mosquito control. To combat this, a new type of insecticide with a novel mode of action needs to be developed. Compounds targeting potassium channels have been developed and analyzed for insecticidal activity. These catechol containing compounds have been shown to be toxic (ca. 200 ng) and selective between mosquito species. Interpretations of the unique selectivity and validation as a novel insecticide will be presented.

### 18 Surveying mosquito larvae in Northern Haiti using high-resolution imagery

Dayana M. Samson, dsamson@med.miami.edu

The effects of the January 2010 earthquake on the transmission of mosquito-borne diseases like malaria and dengue are unknown. This study evaluated how natural and anthropogenic land cover/land use changes in Cap-Haitian and Caracol have influenced mosquito population and the potential for post-earthquake vector-borne disease transmission. The findings will provide new information on how anthropogenic changes due to population growth and expansion of the diverse urban environment affect disease risk.

## Adult/Aerial Control

### 19 Comparison of two styles of adult mosquito exposure cages for testing adulticide applications

Jeffrey Stivers, Jeff@cmcd.org

Two adult exposure cage designs were tested under operational conditions at the Collier Mosquito Control District (CMCD). The cages tested were a "ring" design, developed at the CMCD, using nylon tulle as screening and a "cylinder" cage, developed at the Manatee County Mosquito Control District, using plastic screening. Testing was performed during 4 nights of routine aerial adulticide missions. Two cages of each style were placed at each sample site within the targeted areas. Approximately 20 wild-caught mosquitoes from the same source were placed in each of the cages and provided with sugar water until testing. After testing, the mosquitoes were transferred to clean holding cages, provided with sugar water and held for 24 h. Mortality was determined 24 h post-treatment. Mortality values for each cage style were compared using a paired T test. There was a significant difference ( $P \leq .001$ ) in mortality between the styles, with the "ring" cage demonstrating greater mortality.

### 20 Aerial ULV application of Dibrom against adult mosquitoes in an extreme hot-arid zone

Seth C. Britch, seth.britch@ars.usda.gov, Kenneth J. Linthicum, Robert L. Aldridge, Mark Breidenbaugh, Karl A. Haagsma, Thomas E. Janousek, Jeremy Wittie, Gregory White, Melissa Snelling and Arturo Gutierrez

Aerial ULV pesticide application has been well studied in hot, humid environments, but less is known regarding efficacy in hot-arid environments similar to those occupied by US military personnel where populations of disease-vector or nuisance mosquitoes or sand flies pose significant threats to force health. We placed sentinel mosquitoes in cages on a line of poles in the Naval Air Facility El Centro parachute range located in a hot-arid desert in southern California. Sentinel cages were placed in open sandy areas and within low canopy desert vegetation. Dibrom was applied perpendicular to the cage line in 2 trials for each of 2 nozzle sizes from a USAF C-130H aircraft at ~150 ft altitude at the label rate, and we recorded treated and control sentinel mortality. In general, vegetation reduced mosquito mortality ~10-20%, the smaller nozzle size produced a narrower swath of mortality, and maximum mortality for 3 trials was observed within ~250 ft of the flight line. However, in one trial with winds from roughly the same direction as the flight line, mortality was higher in some vegetated areas, and the mortality swath was >2,000 ft. Results indicate aerial application of Dibrom can be effective in hot-arid environments against mosquitoes, and although vegetation may provide some refuge from pesticide, efficacy can be maintained in the target zone under a variety of wind conditions.

### 21 Re-envisioning mosquito control: development of a robotic multirotor aircraft

Randy Gaugler, gaugler@rci.rutgers.edu, Gregory M. Williams, Scott Crans, Rafael Valentine, Devi Suman and Yi Wang

In 1930, Rutgers made the first spray application by air for mosquito control. Aerial delivery of 8 gal/acre of furnace oil was ridiculed as pie-in-the-sky; but by 1947, a million acres were being treated annually with DDT via aircraft. In 2013, we are challenged to reduce pesticide use. We submit that unmanned aerial vehicles offer potential to achieve this goal by reducing broadcast in favor of targeted applications. We have constructed a prototype multirotor aircraft for <\$6k for

proof-of-concept experiments. Our 6-rotor, carbon fiber, heavy-lift, autonomous robocopter weighs less than 10 kg (FAA compliant) yet is equipped with an array of sensors, including sonar, barometer (altitude hold), magnetometer (direction), gyros (stabilization), 2-axis camera (video downlink), accelerometers, long range digital control, optical flow (position hold), GPS, radio telemetry and more. The critical batteries are highly advanced military-grade lithium polymers. We intend to test the feasibility of the aircraft to contribute to multiple functions including adulticiding, larviciding, larval collection, adult collection, and surveillance. Prototypes for each of these 5 essential mosquito control functions have been developed as we explore the next step in the evolution of aerial platforms.

**22 Aerial larval control with Altosid SR20 applied from an autonomous multirotor aircraft**

Gregory M. Williams, gwilliams@hudsonregionalhealth.org, Scott Crans and Randy Gaugler

Remotely-controlled aircraft have been explored for mosquito control since the early 1970s. More recent attempts utilized expensive unmanned aircraft that were difficult to pilot. Current developments in multirotor helicopter technology have made the concept of autonomous aerial mosquito control applications a reality. We developed a small inexpensive hexacopter and accompanying spray system for autonomous mosquito control missions. We present the results of the first autonomous application of Altosid SR20 to a field site, discuss the design of the aircraft, and explore the future potential of such systems.

**23 The fight over flight: Is airplane spraying keeping mosquitoes down?**

Banugopan Kesavaraju, banu@slcmad.org, Nicholas Delisi and Sammie Dickson

Droplet characteristics of Dibrom, Duet, Zenivex, and Biomist from truck and airplane mounted ULVs were compared in the Salt Lake City area for 4 months. Droplet density for permethrin-based products was higher with offset but was the opposite for Dibrom indicating that offset models for Dibrom do not work well for the area. FLB and Hock spinners were also compared in the field for efficiency in droplet collection which showed that FLB spinners are more efficient.

**24 Optimizing effective aerial spray window in Casper, Wyoming using Anvil® 10+10 against *Aedes dorsalis* & *Culex tarsalis***

John Leman, johnle@cnchd.org and Fran Krenick

Aerial control of *Aedes dorsalis* and *Culex tarsalis* can be challenging under normal conditions. Compound that challenge with mountain meteorology and it's even trickier. This paper will discuss how sequential trapping revealed the peak host-seeking time window for successful control of both species in Natrona County, WY. Often conditions during mosquito activity periods are not conducive to aerial ULV applications. This paper discusses how important it was to be armed with the right timing and proper product characterization for successful aerial application using Anvil® 10+10.

**25 Evaluating VectoBac® WDG as an aerial larvicide for *Aedes aegypti* control**

Catherine Pruszyński, cpruz@keysmosquito.org

VectoBac® WDG is a water dispersible granule formulation of *Bti* that can be aerially applied to treat large populated areas. The Florida Keys Mosquito Control District has been using VectoBac® WDG since 2010 to control for *Aedes aegypti* larvae in containers throughout Key West. In this experiment, aerial application of VectoBac® WDG was conducted once a week over a 5-wk period beginning May 31, 2013, and then repeated bi-weekly until September 5, 2013. Efficacy was evaluated using *Ae. aegypti* adult population data obtained from BG Sentinel traps and ovicup index in treatment and control sites. In this presentation, we discuss the 15 wk of results in the treatment area compared to the control area and to previous years' population trends. Further cost analysis must be performed to determine the minimum effective dosage to achieve reduction.

**26 Global malaria control: Are we doing the right thing? Are we doing things right?**

Manuel Lluberas, lluberas@hdhudson.com

Malaria has been eradicated or reduced and is no longer health or economic burden to a large number of countries. Almost without exception, eradication was reached through active vector control and involving the local population to make their immediate environment less conducive to the proliferation of mosquito populations. More significantly, every country that achieved eradication did so more than 2 decades ago, long before the establishment of many of the current anti-malaria initiatives and without the benefit of a vaccine. While much has been said about the expenditures related to malaria control and the funding shortfalls many programs face, there has been very little said regarding their evaluation. Continued reliance on passive methods like mosquito nets without active mosquito control methods that include IRS, larviciding and adulticiding with ULV sprays will only guarantee malaria's existence. Well into the 21<sup>st</sup> century, public health entomologists continue to wonder why the methods that eradicated malaria from so many countries and have kept it out for decades continue to be overlooked and neglected by the agencies and organizations that promoted and implemented them so aptly early in the 20 century. Examples of well-organized mosquito control programs that directly attack the vector and have been instrumental in eradicating or reducing malaria to a point where it does not overwhelm continuously diminishing public health resources are provided.

## Legislative and Regulatory II

### 27 Mosquito control on federal lands

Bill Meredith, William.Meredith@state.de.us

Updates on the status of the USFWS's proposed "National Mosquito Management Policy for NWRs"; on the status of the AMCA's draft "Federal Lands Mosquito Control Accommodation Act", and on the status of the AMCA's new "Helpful Information to Have or Consider for Mosquito Control on National Wildlife Refuges" guidance document; on the status of the mosquito control situation at Bandon Marsh NWR (OR); plus possibly some updates on other federal lands concerns.

### 27a Pesticide concerns in the Pacific Northwest

Scott Dahlman, scott@ofsonline.org

Presentation will focus on legal challenges to pesticide use in Oregon including pollinator incidents resulting in federal labeling changes, and an investigation in the Triangle Lake area that had statewide and national implications. Oregonians for Food and Shelter (OFS) is a pro-active, member-funded, grass roots coalition which works to protect the rights of natural resource based businesses in Oregon. Our primary focus is protecting those who responsibly use pest management products, soil nutrients and biotechnology from government over-regulation. OFS works to protect the right to use these tools in a legal and responsible manner. We also get involved in a variety of other issues affecting farmers, foresters and urban businesses relating to natural resources.

### 27b USFWS Bandon Marsh Wetland Restoration Project- failure to plan is planning to fail!

James Lunders, vector@jeffnet.org

Brief history of the Bandon Marsh Restoration Project. USFWS and Coos County's response to saltmarsh mosquitoes produced by the project. AMCA, NWMCA's & OMVCA's involvement. What have we learned? What can we do to prepare for next time?

### 28 AMCA Washington conference

Thomas R. Wilmot, twilmot@co.midland.mi.us

The AMCA Washington Conference provides an opportunity for Association members to receive updates on legislative and regulatory issues of greatest concern to mosquito control professionals and to become active participants in American democracy. After an afternoon of discussions and training, AMCA members proceed to Capitol Hill to meet with elected representatives and staff. Only by working together can we most effectively influence regulators and legislators. Please plan to join us at the 2014 Washington Conference, proposed dates May 4-7, 2014.

## Student Competition II

### 29 Heritable variation in the sensitivity of *Anopheles gambiae* to DEET

James D. Ricci, jricc001@ucr.edu

Despite widespread and prolonged use, insensitivity to DEET has rarely been observed in mosquitoes. Also, DEET's mode of action upon mosquitoes is still debated. We demonstrate significant variation in DEET sensitivity in a population of *Anopheles gambiae*, and that this variation can be selected for over time. Continued selective pressures may produce wild populations insensitive to DEET. Genomic analysis of selected lines will reveal genes responsible for DEET insensitivity and its mode of action.

### 30 Attractive toxic sugar bait for biting midge control

Darren S. Snyder, snyderd@ksu.edu

One of the latest trends in mosquito control is the use of attractive toxic sugar bait (ATSB) to reduce adult mosquito populations. Tested here is the ability of ATSB's to kill the biting midge, *Culicoides sonorensis*, a bluetongue, epizootic hemorrhagic disease, and vesicular stomatitis disease vector. The commercial pesticide formulations (percent active ingredient) tested were Talstar® (7.9% bifenthrin), Tempo® (1% cyfluthrin), Suspend® (4.75% deltamethrin), Dragnet® (36.8% permethrin), Platinum® (75% thiamethoxam), QuikBayt® (0.5% imidacloprid), Safari® (20% dinotefuran), Natular® (21.6% spinosad), Ecoexempt® (10% rosemary oil/2% peppermint oil). The time to 90% mortality (LT90) and lethal dose to kill 50% (LD50) were calculated for various concentrations of pesticide (0.01%, 0.05%, 0.10%, 1%, 2% and 3%). In the first set of trials colony midges were fed *ad libitum* sugar and pesticide solutions. In the second set of trials competitive feeding was evaluated and midges were provided clean sugar solution (10% sucrose) in 1 vial and sugar+pesticide solution in another to determine if the pesticide would deter feeding. Talstar® and Dragnet® caused the most mortality (>90% mortality) in the first hour at the lowest concentrations and Ecoexempt® caused the least mortality.

**31 Leaf detritus of native and exotic invasive shrubs alters survival and development of the West Nile virus vector *Culex pipiens* (Diptera: Culicidae)**

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We test the hypothesis that leaf detritus of native and exotic invasive shrubs have asymmetric effects on survival and development of the West Nile virus vector *Culex pipiens*. Differential response of *Cx. pipiens* to the 6 leaf detritus types was observed, with invasive honeysuckle and native blackberry respectively yielding the best and the worst performance. Our findings may be relevant to vector management and our broader understanding of the ecological impact of exotic invasive plants.

**32 Laboratory evaluation of citronella, picaridin, and DEET repellents against *Psorophora ciliata* and *Psorophora howardii***

Jodi M. Scott, bowerj@ufl.edu

Three commercially available repellents: OFF® Active (AI: 15% DEET), OFF® Family Care (AI: 5% Picaridin), and CVS Pharmacy Fresh Insect Repellent (PFIR) (AI: 10 % Citronella) were evaluated to determine the mean protection time provided against the large flood water mosquitoes *Psorophora ciliata* and *Ps. howardii*. CVS PFIR provided the least protection time (2 h and 26 min) followed by OFF® Family Care (3 h and 46 min) and lastly OFF® Active afforded the longest protection (5 h and 41 min).

**33 Effects of larval temperature and nutrition on dengue-1 virus vertical transmission by *Aedes aegypti* and *Aedes albopictus* mosquitoes**

Eva Buckner, eva.buckner@ufl.edu

In certain areas, vertical transmission may allow dengue virus (DENV) to persist in the environment during inter-epidemic periods. In an effort to better elucidate what impact larval ecology has on local DENV vertical transmission rates, I examined the influence of larval temperature and nutrition on DENV-1 vertical transmission in *Aedes aegypti* and *Ae. albopictus* mosquitoes. I found that larval temperature had a statistically significant effect on vertical transmission rates.

**34 Resistance of Puerto Rican *Aedes aegypti* to permethrin, etofenprox, and propoxur**

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Insecticide resistance impacts vector control. Characterizing insecticide resistance in mosquitoes allows for the optimization of control strategies. Resistance to three insecticides was determined for susceptible and resistant (Puerto Rico) strains of *Aedes aegypti*. Groups of 10 females were topically dosed in triplicate. Dose-response curves were generated for each chemical pair and resistance ratios were calculated using the LD<sub>50</sub> values. Resistance ratios were high for the pyrethroids and low for propoxur.

**35 A melting curve PCR assay that can detect the *ace-1* mutation in *Culex quinquefasciatus***

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Insecticide resistance is a threat to public health. Acetylcholinesterase resistance decreases organophosphate sensitivity. A melting curve assay was designed to detect the glycine-serine *ace-1* mutation in *Culex quinquefasciatus*. Allele-specific PCR detected single nucleotide polymorphisms. Three melting curve profiles were observed indicating homozygous or heterozygous genotypes. This tool could be used for early resistance detection, which is critical for management strategies development.

## Advances in Resistance Management in Mosquitoes to Larvicides and Adulticides I

**36 Resistance and resistance management in mosquitoes: Where to go from here?**

William G. Brogdon, wgb1@cdc.gov

Insecticide resistance is one of the critical major threats not only to multi-national vector-borne disease control programs but also to both local and regional mosquito control activities. The most serious specific problem is the widespread emergence of resistance to the pyrethroid class of insecticides. The high costs of managing pyrethroid resistance through rotation to other insecticide classes are producing crises in large programs. Moreover, the large bednet distributions that are dependent upon to reduce the incidence of malaria and other diseases suffer from the fact that only pyrethroids are permitted for use on LLINs. The single factor operating in our favor is that pyrethroid resistance is both highly focal and, with the application of appropriate management techniques, unstable. A combination of detoxification and target site mechanisms is generally required for the more intense levels of resistance. In certain instances, a move to the proper insecticide class can directly attack particular resistance mechanisms. This means that the key to economically sustainable resistance management is efficient, but thorough resistance surveillance using simple, inexpensive tools such as the bottle bioassay in its standard and intensity detection formats. Especially exciting are recent findings that appropriate resistance management techniques may be leading to focal restoration of pyrethroid susceptibility.

**37 Global status of resistance and resistance management to biorationl larvicides for mosquito control**

Tianyun S. Su, tsu@wvmvcd.org and Min Lee Cheng

Biorational larvicides based on microbial derivatives and IGRs play increasingly important roles in mosquito control operations due to their high efficacy, low impact on non-target species and environmentally compatible profiles. Thus far, lack of resistance to whole *Bti* has been documented since 1980s. However, resistance to individual toxins of *Bti* has also been well elucidated since 1990s. Resistance to *Bacillus sphaericus* (*Bsph*) in field mosquito populations has been documented in many parts of the world during the past decade; behavioral, biochemical and molecular mechanisms involved in resistance development to different geographical isolates of *Bsph* were studied worldwide. The strategies of resistance management to *Bsph* have been developed for resistance prevention and susceptibility restoration. Recently, mosquito resistance and cross-resistance to spinosad have been studied under laboratory conditions. Research on resistance management strategies to spinosad is being explored. Resistance management to IGRs such as juvenile hormone analogs and chitin synthesis inhibitors drew some attention back in the 1970s and early 1980s; however, since then subsequent studies and field monitoring of IGR resistance have been meager. Recommendations for resistance prevention and susceptibility restoration are suggested according to modes of actions of different biorational larvicides.

**38 Mosquitocidal Bts and their potential for *B. sphaericus* resistance management**

Margaret C. Wirth, mcwirth@ucr.edu, William E. Walton and Brian A. Federici

*Lysinibacillus sphaericus* (formerly *Bacillus sphaericus*) is a toxin-producing bacterium used in formulated insecticides to control mosquito larvae. The benefits of *L. sphaericus* are its specificity and longevity in larval habitats with high organic content. Unfortunately, *L. sphaericus* can rapidly select for insecticide resistance in frequently treated larval populations. In fact, field cases of resistance were detected in as little as 2 yr after implementation of use, and cases of *L. sphaericus* resistance have been documented in *Culex* populations throughout the world. The rapid evolution of *L. sphaericus* resistance is a consequence of the single-site action of the bacterial toxin. *Lysinibacillus sphaericus* has 2 toxin proteins, BinA (toxic component) and BinB (binding component); both are required for maximum toxicity. Because *L. sphaericus*' mode of action depends on a single receptor protein in the midgut epithelium, a GPI-anchored alpha-glucosidase, any disruption in binding of Bin to this target inevitably leads to high levels of insecticide resistance. The multi-toxin bacterium *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) has proven to be highly refractory to selecting resistance. We will present data showing that increasing toxin complexity in *L. sphaericus*-based larvicides by combining it with *Bti* toxins is an effective strategy to delay the evolution of *L. sphaericus* resistance.

**39 Susceptibility status and resistance development potential of insect growth regulators [IGRs]**

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Insect growth regulators (IGRs) such as methoprene, pyriproxyfen, and diflubenzuron are valuable public health mosquitocides because they have a completely different mode of action than the common organophosphates and pyrethroids. The judicious use of IGRs can reduce selection pressure on OPs and pyrethroids and significantly prolong their effectiveness. However, insecticide resistance to some of these IGRs has been reported: 1) the resistance potential of *Culex quinquefasciatus* against methoprene and diflubenzuron; 2) laboratory selected resistance to diflubenzuron in *Aedes aegypti*; 3) high levels of methoprene resistance in *Ochlerotatus nigromaculus* in central California; and 4) methoprene resistance in *Aedes taeniorhynchus* on Sanibel Island in Florida. A signature problem with IGRs is that they do not kill all mosquito larvae exposed to lethal concentrations. Some pupae may survive to neonate adults that die on the water surface. The response to lethal concentrations is not a simple "dead/not dead." In other words, the outcomes are not binomial and standard probit analysis is not adequate to fully analyze IGR dose response data. A user-friendly, multinomial probit model computer program is sorely needed. This presentation will briefly summarize the current susceptibility status of IGRs and will focus on specific needs for further research.

**40 Susceptibility of *Aedes vexans* populations after 30 years of mass applications of formulations based on *Bti***

Nobert Becker, norbertbecker@web.de and Mario Ludwig

The mosquito control programmes in Germany, particularly in the Upper Rhine Valley, are solely based on the application of various formulations of *Bacillus thuringiensis* var. *israelensis* (*Bti*). Since 1981, up to 20,000 ha of mass larval floodwater mosquitoes (mainly *Aedes vexans*) sites are treated by helicopters or ground application using more than 4,000 tons of sterilized *Bti*-based formulations. In time intervals of 3 yr at 6 locations in regular *Bti* treated areas (Upper Rhine Valley) and 3 untreated areas at Lake Constance soil samples -- always at the same locations where *Aedes vexans* is by far the dominant species-- have been taken and flooded to hatch larvae. In bioassays following WHO guidelines, the mosquito sensitivity of the various populations towards *Bti* has been assessed by log-probit analyses. By comparing log-probit-analyses by statistical evaluations it could be shown that neither LC<sub>50</sub>- values and slopes nor probit-lines of bioassays of the larvae deriving from treated and untreated areas showed significant differences. These results have been confirmed by resistance ratios which were 0.90 - 0.99 in all tests. The results give evidence, that no resistance phenomena in *Aedes vexans* have yet developed in areas of the Upper Rhine Valley treated with *Bti*. This unique feature of *Bti* will be discussed.

**41 *Bti*: a powerful tool for resistance management and synergism to other larvicides**

Min Lee Cheng, mcheng@wvmvcd.org and Tianyun S. Su

*Bti* has been playing an important part in mosquito control operations since its discovery and development in 1980s because of its high potency, quick larvicidal activity, target specificity, and benign environmental profile. Among the 4 endotoxins (Cry 4A, 4B, 11A and Cyt1A) produced by *Bti* during sporulation, Cyt1A is the key factor for larvicidal activity enhancement and resistance prevention. *Bti* significantly enhances larvicidal activity of *B. sphaericus* (*Bsph*) against *Aedes aegypti* in laboratory studies; it kills *Bsph*-resistant *Culex quinquefasciatus* under laboratory and field conditions. *Bti* prevents resistance development and restores susceptibility to *B. sphaericus* in *Cx. quinquefasciatus* under laboratory conditions. Recent laboratory studies indicate that *Bti* enhances larvicidal activity of spinosad and kills mosquito larvae which are highly resistant to spinosad. It is worthwhile exploring the potential role of *Bti* in resistance management against spinosad. Interestingly, *Bti* also partially restores susceptibility to the juvenile hormone analog methoprene in a field *Aedes nigromaculis* population which has developed high level of resistance to methoprene after long-term application of methoprene products in the field. *Bti*, a powerful tool for resistance management and its synergism to other larvicides, deserves our attention to preserve its longevity for long-term benefits in mosquito control.

## Disease Vector I

**42 A decade (2004-2013) of WNV activity in Orange County, California: from patterns to predictions?**

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After Orange County's first West Nile virus (WNV) outbreak in 2004, levels of WNV activity have oscillated between years of subsidence (2005-2007; 2009-11) and recrudescence (2008 & 2012) on an apparent 4-yr cycle. This presentation will discuss some of the spatial and temporal patterns of WNV epidemiology in the county during the past 10 yr. GIS-based analyses of WNV activity patterns and various socioeconomic, biologic, and environmental factors will be discussed as a potential aid in focusing mosquito control efforts to reduce human cases of WNV in the future.

**43 Development of a spatially-tiered warning system for WNV activity with application to county-level control policy**

Christopher N. Mores, cmores@lsu.edu, Justin K. Davis, Carrie Manore and Rebecca C. Christofferson

West Nile virus is a continuing public health hazard, with a significant upsurge in human cases during 2012. Since its introduction in 1999 and subsequent spread across North America in 2001-2002, the virus has been maintained within an enzootic cycle of birds and associated *Culex* mosquitoes. While efforts have been made to anticipate increases in transmission, predictions have been problematic. Thus, we partnered with regional (mosquito abatement), state health and national agencies (NOAA, CDC), to collate and integrate data into a model with the potential to inform policy and mitigation strategies. We developed an early warning system, which can accurately fit human WNV case data. Our preliminary model indicates that environmental variables (minimum temperature, land cover) and bird richness are important factors in being able to predict the potential for elevated WNV activity in the human population. Further, we present the framework for regional and spatially explicit models to address more specifically focal transmission questions.

**44 La Crosse encephalitis virus infected *Aedes* species in Tennessee**

Katie Westby, kmwest2@ilstu.edu, Charissa Fritzen, Dave Paulsen, Reid Gerhardt, Carl Jones and Abelardo Moncayo

La Crosse virus (LAC) is a mosquito-borne virus and a major cause of pediatric encephalitis in the US. LAC emerged in Tennessee and other states in the Appalachian region in 1997. We investigated the potential roles of the native mosquito vector, *Aedes triseriatus* (Say), and 2 recently introduced mosquito species, *Ae. albopictus* (Skuse) and *Ae. japonicus* (Theobald), in LAC transmission in an emerging disease focus in Tennessee. Mosquitoes were collected using multiple trapping methods specific for *Aedes* mosquitoes at recent case sites. Mosquito pools were tested via RT-PCR to detect multiple Bunyamwera and California serogroup viruses including LAC. A total of 63 mosquito pools were found positive; mostly from adults reared from egg collections, demonstrating transovarial transmission in all 3 species. Maximum likelihood estimates varied among the 3 vector species from 3.55 for *Ae. triseriatus*, 2.82 for *Ae. albopictus*, to 0.63 for *Ae. japonicus*. This may be related to the length of time the species have been present in the area, with *Ae. triseriatus* as the native species and *Ae. albopictus* and *Ae. japonicus* as recent invaders. We conclude that *Ae. triseriatus* and *Ae. albopictus* are important LAC reservoir species and that *Ae. japonicus* may also be involved in virus maintenance as well.

**45 RAMP assay biosafety: flaws and fixes**

Kristen L. Burkhalter, ktb3@cdc.gov and Harry M. Savage

We evaluated the ability of the Rapid Analyte Measurement Platform (RAMP) mosquito grinding buffer to inactivate West Nile virus (WNV) by subjecting mosquito pools spiked with serial dilutions of WNV and ground in 1-ml RAMP buffer to incubation intervals ranging from 15-60 min. At each time point, an aliquot was removed and serially diluted in BA-1 cell culture media to stop the inactivation process. Each BA-1 sample was tested for viable virus using Vero 6-well cell culture plaque assay and observed for plaques; results were also compared to the plaque production of serially diluted WNV in BA-1. We observed only partial inactivation of WNV, evidenced by viral plaques produced in high-titered samples incubated in RAMP buffer. Concerned for the safety of workers who may be using the RAMP assay in low-level bio-

containment facilities, we developed an alternate sample homogenization protocol that ensured complete WNV inactivation without compromising the performance of the RAMP assay.

**46 WNV preparedness and responsiveness from data driven models**

Rebecca C. Christofferson, rcarri1@lsu.edu, Helen J. Wearing, David F. Westneat and Christopher N. Mores

West Nile virus (WNV) re-emerged as a public health threat in 2012 after several years of quiescent transmission. Studies into the mechanisms of such a high human case rate are reliant on retroactive investigations, as the enzootic cycle of the virus remains relatively uncharacterized. Thus, tangential transmission into human populations is difficult to predict without appreciation of the forces that pull together the enzootic cycle and human populations. Accordingly, we investigated the use of coincident, domestic and peri-domestic House Sparrow populations (*Passer domesticus*) to theoretically investigate possible drivers of the 2012 season. Specifically, we implemented a stage-structured model that takes into account different dynamics and life traits of juvenile versus adult sparrows, and we investigated the possibility of environmental factors as contributors to transmission changes. In addition, we will demonstrate the ability of this model to assess different control methods and timing.

**47 Experimental infection of *Amblyomma americanum* with Heartland virus (Bunyaviridae: *Phlebovirus*)**

Marvin S. Godsey, mjg9@cdc.gov, Kristen L. Burkhalter and Harry M. Savage

Heartland virus (HRTV) was first recognized when it was isolated from 2 residents in northwest Missouri who were hospitalized during 2009 with severe febrile illness. Field studies during 2012 implicated *Amblyomma americanum* ticks as a probable vector. HRTV was isolated from nymphal ticks, but not from larvae or adults. To understand the role of *A. americanum* as a vector of HRTV we attempted to infect immature ticks using an immersion technique. In preliminary trials, nymphs and larvae were immersed in virus, and then held for 21 d before testing for virus. In a later trial, larvae were immersed, then fed on a rabbit, allowed to molt to the nymphal stage, and the resulting nymphs tested to assess transstadial transmission of virus. Immersion followed by a rabbit feed greatly enhanced HRTV replication. The ultimate goal of the study is to test the ability of infected larval ticks to pass the virus transstadially to nymphs, then on to the adult stage, and for infected females to transmit virus vertically to their progeny.

**48 A comparison of VecTest™ and VectorTest™ in southeastern Virginia during years of West Nile virus and abundant eastern equine encephalitis activity**

Jay P. Kiser, jkiser@suffolkva.us, Charles F. Abadam and Karen I. Akaratovic

Because of funding cuts in the spring of 2012, Virginia state-run laboratories ceased to provide RT-PCR testing of mosquito pools for eastern equine encephalitis (EEE) and West Nile virus (WNV). Alternative testing methods were sought and most of southeastern Virginia chose to use VecTest™, an in-house antigen assay capable of testing both viruses of interest, during the 2011 and 2012 mosquito seasons. During this time, 165 EEE and 75 WNV positive mosquito pools were detected by this test. In the fall of 2012, VecTest was no longer produced and the region started using VectorTest™, a similar in-house antigen assay, for the fall of 2012 and entire 2013 season. During this time, 80 EEE- and 28 WNV-positive mosquito pools were detected by the VectorTest. Detection of high viral activity provided opportunity to compare and contrast these 2 testing methods. All positive pools were validated via RT-PCR, which confirmed VecTest and VectorTest. EEE-positive pools of *Culiseta melanura* were 99% and 97% positive, respectively; WNV positive pools of *Culex pipiens* were 94% and 100% positive, respectively. The confirmation rates of VecTest and VectorTest showed a significant difference in WNV positives pools of *Cs. melanura*, which were 48% and 12% respectively. Results with these alternative testing methods appear accurate in detecting EEE in *Cs. melanura* and WNV in *Cx. pipiens*; however, both testing methods showed high numbers of false positives for WNV in *Cs. melanura melanura*.

**49 Update on Heartland virus (Bunyaviridae: *Phlebovirus*), a human pathogen transmitted by ticks**

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In June 2009, 2 men residing on geographically distant farms in NW Missouri were admitted to a hospital with ehrlichiosis-like symptoms. A new *Phlebovirus* was isolated from both patients and named Heartland virus. During the summer of 2012, we conducted field studies in NW Missouri and detected 10 RT-PCR virus positive pools composed of nymphs of *Amblyomma americanum*. The infection rate in *A. americanum* nymphs at a patient property were 1:500 over the study period. In 2013, we conducted field studies in NW Missouri at sites that yielded virus in 2012, and at properties owned by 2 new human Heartland cases. Infection rate data from the 2013 tick field studies will be presented. In addition, a general update will be presented on a broad array of CDC studies on wild and domestic animal serology, development of a laboratory animal model of transmission, application of the immersion technique to infect ticks, and CDC's human surveillance and testing program.

**50 Effect of environmental temperature on the vector competence of mosquitoes for Rift Valley fever virus**

Michael J. Turell, michael.j.turell@us.army.mil and William C. Wilson

Environmental temperature has been shown to affect the ability of mosquitoes to transmit numerous arboviruses and for Rift Valley fever virus (RVFV) in particular. We evaluated the effect of incubation temperatures ranging from 14°C -26°C on infection, dissemination, and transmission rates for *Culex tarsalis* and *Aedes taeniorhynchus* allowed to feed on

hamsters infected with RVFV. Engorged mosquitoes were randomly allocated to cages and placed in incubators maintained at 14°, 18°, 22°, or 26°C. While infection rates detected in *Cx. tarsalis* increased with increasing holding temperature, holding temperature had no effect on infection rates detected in *Ae. taeniorhynchus*. However, for both species, the percentage of mosquitoes with a disseminated infection after specific extrinsic incubation periods (7, 10, 14, 17, or 21 d), increased with increasing incubation holding temperature, even after adjusting for the apparent increase in infection rate in *Cx. tarsalis*. The effects of environmental factors, such as ambient temperature, need to be taken into account when developing models for viral persistence and spread in nature.

## 52 Ideals and challenges faced by professionals in mosquito research and control in Africa

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A thorough knowledge of the vectors and diseases they cause is the foundation for the development and implementation of any vector management strategies. Much can be learned from a review of the historical context of vector-borne diseases. A perspective of the evolution of our knowledge on the role of arthropods in the transmission of diseases and the implication this has had on research and control will provide a view of what is known and what control has actually worked. Despite ongoing control efforts, diseases transmitted by mosquitoes, such as malaria, filariasis, dengue, and other arboviruses continue to pose an enormous global health burden. The intensity of transmission and insecticide resistance are key components which provide information on the risk of epidemic development and also base line information for planning vector control interventions. Such information is not readily available for real time-decision making process. To address these ideals and challenges that professionals in mosquito research and control face today and what needs to be done in the future, a professional body, the Pan African Mosquito Control Association (PAMCA) was established.

## 53 Field evaluation of volatile chemical cues from a non-preferred host of the malaria mosquito, *Anopheles arabiensis*, and their potential as novel repellents

Kassahun T. Jaleta, kassahun.t78@gmail.com, Sharon R. Hill, Habte Takie, Emiru Seyoum, Göran Birgersson and Rickard Ignell

Host preference analysis of the malaria vector, *Anopheles arabiensis*, including the abundance of potential hosts and the blood-meal content of wild-caught mosquitoes show that humans, cattle, goats and sheep are preferred hosts, whereas chickens are not preferred. To research the mechanism underpinning this host discrimination in *An. arabiensis*, volatile headspace collections from the preferred and non-preferred hosts were made, and biologically active compounds identified using combined gas chromatography and electroantennographic detection (GC-EAD), and combined gas chromatography and mass spectrometric (GC-MS) analyses. This analysis revealed both generic as well as host-specific compounds. To test the potential of compounds emitted by the non-preferred host in manipulating the host-selection behavior of *An. arabiensis*, a field experiment was conducted in the Wama Kussaye village in western Ethiopia. Results obtained in this study showed that a significantly lower number of mosquitoes were caught in traps baited with select generic vertebrate compounds, as well as in traps baited with 1 of the tested non-preferred host volatiles. The results of this study implicate specific volatiles emanating from non-preferred host as allomones, which suggests that these volatiles are repellent to host-seeking *An. arabiensis*.

## Latin America Symposium I

### 54 Entomological surveillance of dengue-infected *Aedes aegypti* in the home environment: a population based study in two regions of southern Mexico

Ewry Zarate-Nahon, AEZNX1@hotmail.com, Rocio Ramirez-Jimenez, Jose Legorreta, Arcadio Morales, Elizabeth Nava, Miguel Flores, Sergio Paredes, David Gasga, Alejandro Balanzar, Esteban E. Diaz Gonzalez, Alejandro Gaitan Burns, Ana K. Espinosa, Alejandra Montemayor Puente, Rosa María Sánchez Casas, Ildefonso Fernandez-Salas and Niel Andersson

Multiple dengue virus serotypes circulating concurrently increase the risk of the severe form of the dengue. There is co-circulation of DENV-1, DENV-2 and DENV-4 in Mexico's Guerrero State which in 2012 confirmed 2,606 cases of dengue fever and 1,899 dengue hemorrhagic fever cases, including 8 deaths. A total of 7,166 household surveys assessed household knowledge about dengue fever as part of a large scale cluster randomized trial in the coastal regions of the state. In a random sub-sample of these households (596 in Costa Grande and 595 in Acapulco), a skilled operator collected adult mosquitoes (Prokopack aspirator) in August/September and again in the same households in November/December. Of 2,585 *Aedes aegypti* collected and tested for Costa Grande and 2,991 in Acapulco, RT-PCR and PCR identified 2 DENV-2 positive mosquitoes, in the second cycle in Acapulco and Costa Grande, respectively. This confirms the low yield of population based entomological surveillance; a preferable strategy would focus on homes with a history of dengue.

### 55 The mitochondria as a model for evaluating mechanism of action to compounds with potential insecticidal activity on *Aedes aegypti* (Diptera: Culicidae)

Viviana A. Salamanca, nana18103@gmail.com, Jonny E. Duque and Stelia C. Mendez

Mitochondria are a good model for evaluating a pesticide's mechanism of action. The aim of this study was to establish the parameters of mitochondrial bioenergetics of *Aedes aegypti*. We used larval *Ae. aegypti* between III and IV instars to isolate mitochondria. The larvae were fragmented using Van Potter homogenizer and the mitochondria were isolated by



differential centrifugation. We evaluated parameters of state 3, state 4, ADP/O ratio, and respiratory control. The mitochondria were analyzed in presence of 2 substrates independently, malate-glutamate and succinate. In the presence of malate-glutamate, the respiratory control coefficient was 3.91, ADP/O ratio of 2.5, state 3 was 56.7 and state 4 was 6.84 nmol of oxygen consumed/min/mg of protein. In presence of succinate, the mitochondria present respiratory control coefficients of 4.05, ADP/O of 1.95, state 3 of 54.3 and state 4 was 5.73 nmol of oxygen consumed/min/mg protein. The mitochondrial activity was evaluated in the presence of inhibitors of the mitochondrial respiratory chain: rotenone, malonate, antimycin A, cyanide, and oligomycin. It was found that mitochondria of larvae show similar profiles to mitochondria of mammals. This methodology can be used as a model for evaluating the mechanism of action to compounds with potential insecticidal activity.

**56 Susceptibility of *Aedes aegypti* from Acapulco and Merida to insecticides employed for vector control in Mexico**

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The spread of insecticide resistance of *Aedes aegypti*, particularly to pyrethroids, is a major concern of the Mexican dengue control program. We report the first results of a baseline analysis of the susceptibility status of *Ae. aegypti* populations from 2 dengue-endemic Mexican cities: Acapulco, Guerrero (neighborhood Ciudad Renacimiento), and Merida, Yucatan (20 neighborhoods). Mosquito specimens were obtained from cross-sectional collections with ovitraps displayed in residential premises within both study sites during the rainy season of 2012. Each population was evaluated against different insecticide chemical groups used by the Mexican vector control program with the CDC-bottle method: pyrethroids (permethrin,  $\alpha$ -cypermethrin), carbamates (propoxur), and organophosphates (chlorpyrifos) and the diagnostic doses and diagnostic times were in accordance to the CDC guidelines. The knockdown (KD) effect was recorded for pyrethroids. The 24-h mortality was recorded for all the different insecticide chemical groups. The KD of  $\alpha$ -cypermethrin (average 96%) was higher than the KD of permethrin (average 33%) in all the populations from Acapulco and Merida. The *Ae. aegypti* population from Acapulco showed complete resistance to permethrin but showed susceptibility to  $\alpha$ -cypermethrin; only 2 of 20 populations studied from Merida were susceptible to permethrin (90% showed resistance), while 50% were susceptible to  $\alpha$ -cypermethrin. All populations from Acapulco and Merida evaluated were susceptible for propoxur and chlorpyrifos.

**57 Circadian cycle effect on permethrin resistance in *Aedes aegypti* (L.)**

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Current studies in insects have shown susceptibility to toxic agents in a circadian cycle-dependent manner, because of the biochemical processes that are involved in its metabolism. The goal of this study was to determine the effect of circadian cycle on resistance to permethrin in *Aedes aegypti* populations. Samples of immature stages of *Ae. aegypti* from the field, were collected from 2 locations in Vergel, Yucatan, Mexico. Rockefeller and New Orleans susceptible strains were used to compare results obtained with field strains. Female mosquitoes of the F1 generation were subjected to changes in photoperiod, 0 h light: 24 h dark (0:24). Mosquitoes used as control had similar characteristics to those previously described but with photoperiod 12:12. The bottle bioassay was used to determine knockdown concentration ( $KC_{50}$ ), lethal concentration ( $LC_{50}$ ), resistance ratio at 1 h of exposure ( $RRKC_{50}$ ), and at 24 h ( $RRLC_{50}$ ) in field populations and susceptible strains. The results showed alteration of parameters described above between 12:12 vs 0:24 as well as the enzymes alpha-esterases and GSTs, both mechanisms involved in metabolic resistance. *Kdr* Ile1, 016; mutation was present in the field population but not associated with the circadian cycle.

**58 A new ULV spraying technique to increase mortality of house environment *Aedes aegypti* populations: hose attached to nozzle of vehicle-mounted ULV generator in Chetumal, southern Mexico**

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ULV treatments using vehicle-mounted generators are extensively used in Latin American countries to control adult *Aedes aegypti* populations. However, many factors hamper insecticide droplets from falling inside households to control this endophilic mosquito. We decided to attach a high pressure 20-m long hose to a generator nozzle in order to place insecticide mist directly into house indoors and outdoors. The first test was conducted in 50 houses in Chetumal, southern Mexico. Cages with 20 female *Ae. aegypti* were placed in porches, living rooms, and backyards. Water-based permethrin 10% was applied at a droplet size of 15 microns and flow rate of 416 ml/min. The hose was designed and manufactured by London Foggers Inc., Minneapolis, MN. Householders stayed away during spraying and returned 30 min after. Each house was sprayed for 30 sec to 2 min, depending on the number of rooms and backyard size. Mean percent mortality after 1 h resulted in 91.4%, 89.2%, and 66.5% of porch, living rooms, and backyards, respectively, with an overall mean of 82.4%. After 24 h, mortality data showed 99.9%, 99.8%, and 92.8%, with a pooled mortality mean of 97.5%. Our results indicate that this is a very promising alternative to improve household control of adult *Ae. aegypti* populations

**59 Field comparison between a new ULV spraying technique and traditional vehicle-mounted ULV generator to control household *Aedes aegypti* populations: Chetumal, southern Mexico**

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Houses, both indoor and outdoor areas, are preferred resting places for the dengue fever mosquito *Aedes aegypti*. Adult suppression is mainly conducted using vehicle-mounted ULV generators; however, low mortalities are frequently observed because house barriers do not allow enough penetration of insecticide droplets. Effectiveness of a high-pressure hose attached to a ULV generator to spray directly inside households was compared against a street located vehicle-ULV generator. Three groups of 50 houses were used and received the following treatments: hose ULV system, traditional truck ULV generator, and an untreated control area. Cages with 20 female *Ae. aegypti* were placed on porches, in living rooms, and backyards. Permethrin 10% water-based at 416 ml/min flow rate with a 15  $\mu$  diameter was applied in both ULV systems. After spraying, effectiveness to reduce *Ae. aegypti* populations were determined by counts of adults using a CDC backpack aspirator and oviposition activity with ovitraps during 7-days. Overall, the hose ULV treatment was more effective at reducing mosquitoes during the first days than the truck ULV generator. After 7-days, a mean *Ae. aegypti* house infestation rate of 46.8% was produced by the hose ULV versus 54.1% and 65.8% of truck ULV and control area, respectively. Results were biased because larviciding activity by government program was started 3 days post-treatment.

**60 Insecticide treated windows nets and water tank lids for *Aedes aegypti* (Diptera: Culicidae) control in Girardot, Cundinamarca, Colombia**

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Girardot is a dengue endemic city because of its ecological conditions and dynamic *Aedes aegypti* population. It was found that the most productive sites for the immature forms of the dengue mosquito vector are low tanks and "albercas" (small pools) (>88%). Despite the fact that people recognize the insect and the symptoms of the disease, this community has no perception of risks. Because the traditional control measures are not permanent and constant, this responsibility lies basically on the municipal health authorities and are primarily based on the use of larvicides. The national guidelines seek a greater involvement for the control of this insect community, through the design and implementation of long-lasting insecticide-treated nets. A total of 3,483 nets were installed on windows and doors in 958 homes of 10 randomly selected intervention clusters (neighborhoods with 90-100 houses) to be compared with 10 control clusters. Bioassays under controlled laboratory conditions were used to evaluate the persistence of the insecticide using different washing methods. Field bioassays were also conducted to evaluate the net efficiency. A public poll was taken in the communities where the study was conducted to understand the community's perception of the study and attitude about taking it to other neighborhoods. This dengue fever study has benefited the community by creating jobs and providing economic support, which has been widely accepted by the community. This acceptance and these benefits are important because this is the first dengue fever prevention strategy implemented in Girardot, Colombia.

**61 Clinal variation in *Aedes aegypti* (L.) in the state of Veracruz, Mexico**

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In 2012, Veracruz State had the most cases and deaths associated with infection of dengue virus (25% of the cases and 42 deaths) in Mexico. *Aedes aegypti* in southern Veracruz is less competent to DENV infection than some other varieties. The objective of this study was to determine if the subspecies *Ae. aegypti formosus* exists. We collected *Ae. aegypti* and *Ae. albopictus* larvae and pupae in 7 cities of Veracruz in August 2012 and obtained a representative sampling of *Ae. aegypti* eggs for analysis. The tergites of 50 mosquitoes from each collection were analyzed (applying McClelland's method), further revealing the variety *queenslandensis*. Five patterns of *Ae. aegypti aegypti* were found; the majority were the H pattern (83%). Furthermore, 4% were *Ae. aegypti formosus*. After checking the genetic flow of 70 mosquitoes for Dicer-II, Argonaut II and ND4 genes, 20% of our samples had a change of base in a diagnostic single nucleotide polymorphism site of Dicer-II (4327) that belongs to the African subspecies *formosus*. In ND4, our forms belong to 6 forms previously recorded in other studies. Our study suggests the existence of *Ae. aegypti formosus* in northern Veracruz.

**62 Infectivity capacity of nematodes *Romanermis iyengari* and *Strelkovimermis spiculatus* on four types of water of breeding sites of mosquito larvae *Culex quinquefasciatus*.**

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With the objective of determining the infectivity capacity of *Romanermis iyengari* and *Strelkovimermis spiculatus* in larval *Culex quinquefasciatus*, 2 experiments were set up with each species of nematode. Each experiment evaluated 12 treatments, obtained by the combination of three doses (10, 15 and 20 nematodes per larva), with 4 types of water (distilled, contaminated stream, river, and lagoon). One experiment was established for each nematode species. The nematode *R. iyengari* caused a percentage of parasitism (PP) that varied from 52.5 to 100% and MI of 0.95 to 14.02 nematodes per larva; whereas in *S. spiculatus*, the PP varied from 64.38 to 100% and MI from 0.98 to 12.95 nematodes per larva. In the contaminated stream water, the low PP values (57.5 and 64.38%) and MI (0.95 and 0.98) with the dose of 10

nematodes per larva caused by *R. iyengari* and *S. spiculatus*, respectively. In the lagoon water, high PP (100%) and MI (12.95 and 14.02) with the dose of 20 nematodes per larva was caused by *S. spiculatus* and *R. iyengari*, respectively. These results indicate that the use of *R. iyengari* or *S. spiculatus* in a program of integrated management, applying the dose of 10:1 or 15:1 nematodes by larva, may be worthwhile.

**63 DENV-infected *Aedes aegypti* mosquitoes from Quintana Roo, Mexico. Early detection of serotype 4 in field-collected mosquitoes**

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The aim of this study was to identify DENV-infected *Aedes aegypti* mosquitoes in the field from the municipalities Benito Juárez and Othon P. Blanco, Quintana Roo State, Mexico. Mosquito collections were made in 569 households with CDC backpack aspirators and yielded 2,144 females from both localities. They were homogenized separately and 10 mosquito pools were formed in order to process them by RT-PCR. Five (2.27%) out of 220 pools were positive for DENV. Fifty mosquitoes belonging to these pools were processed individually by RT-PCR for serotyping. Six mosquitoes were positive for DENV-2, 1 for DENV-1 and 1 for DENV-4. Serotype 4 had not been reported by Health Department of Quintana Roo state during 2012, but it was detected in the next year, 10 wk after our mosquito collections. The mean numbers of females per household and mosquito infection percentage were  $3.77 \pm 5.71$  and 0.4%, respectively. Interestingly, most (49%) females were captured in only 10% of households, identifying evident aggregation sites in their spatial distribution. This study supports the implementation of infected mosquito field collections as part of dengue surveillance programs, which can be used as an early warning system.

**64 Vector competence to DENV-2 among *Aedes aegypti* (L.) collections from the state of Veracruz, Mexico**

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Dengue has become one of the major arboviral diseases in the world constituting an important public health problem in 2012. The Mexican health system reported more than 50,000 cases of dengue, and 35% of these cases had hemorrhagic manifestations. Twenty-five percent of all the cases occurred in Veracruz State. It has been previously shown that *Aedes aegypti* in southern Veracruz is a less competent vector for dengue viruses. In order to evaluate the level of vector competence among mosquitoes from Veracruz State, we collected *Ae. aegypti* larvae and pupae in 7 cities of Veracruz in August 2012 and obtained a representative sampling of *Ae. aegypti* eggs for analysis. Mosquitoes of the F2 generation were orally infected with DENV-2 Jamaica 1409, after 14 days post infection the midguts and salivary glands of the infected mosquitoes were collected. The infection titer in the different tissues was obtained by plaque assay. The importance of this study was to have a better understanding of the mechanisms of infection within the mosquito that can lead to more competent mosquitoes where the virus can replicate and later be transmitted to susceptible human hosts. The fact that the populations have differences in this regard could explain the distribution of the cases to some extent.

**65 Vertical transmission of dengue virus in immature forms of *Aedes aegypti*, collected in Cancún, México**

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Vector control programs in Mexico have failed to alert or prevent dengue epidemics. They also do not include routine monitoring of immature stages and data on vertical transmission in Mexico is almost nonexistent. The objectives of this study were to: 1) detect and serotype dengue from immature stages of field collected *Aedes aegypti* by RT-PCR; 2) determine the spatial distribution of infected samples; and 3) estimate the minimum infection rate. We sampled 10,605 specimens from 977 containers in 326 homes and processed 91 pools of mosquitoes. RT-PCR was able to detect 3 pools positive to DENV-2. Interestingly, all 3 corresponded to a small cluster of homes evidencing a vertical transmission "hot spot". We determined a minimum infection rate of 0.28. This is the first report in Mexico of DENV detected directly from immature forms. This study demonstrates it is possible to detect dengue in immature forms and highlights the need to reformulate larval control programs in southeastern Mexico.

## **US Military Vector Control Research and Development: Ten Years of Translational Research in the Deployed Warfighter Protection (DWFP) Program I**

**66 US Military entomology research overview**

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Coordinated through the Armed Forces Pest Management Board, the US Military leads research, development, testing, and evaluation activities focused on "leap forward" technologies to achieve dramatic improvements over status quo capabilities. With a clear goal to ensure deployed forces are protected by the most effective disease vector control tools,

the team is focused on delivering:

1. New insecticidally active ingredients, synergists and formulations for vector control
2. Replacements or supplements to permethrin as a military uniform/textile insecticidal treatment
3. New or improved active ingredients and formulations for spatial repellents
4. Improved vector surveillance systems and devices
5. Improved vector control techniques

The symposium will showcase highlights of the current research funded through the Deployed Warfighter Protection Program now celebrating its 10th anniversary.

#### 67 **The legacy of the USDA ARS-DoD Partnership**

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At first consideration, the missions of the Department of Defense (DoD) and the Department of Agriculture (USDA) might seem to have very little to connect them logically. DoD concerns itself with the security of the country as a whole, with all the accoutrements of weapons, transport, and strategy. USDA, on the other hand, was the first of the science-based departments, created in recognition of how the advancement of agriculture could develop a new nation. All nations have had departments of "defense" since very ancient times;- the making of and defense from war has been a part of humanity from the beginning. Systematic advancement of agriculture is a relatively recent trend, especially combined with experimental biology. One of the American miracles was to recognize how much applied biology as agriculture could contribute to the military. To a large extent, the process has been reciprocal; some of the major advances in agriculture have depended on the military. Vector control is only one aspect of this relationship, but it provides a lens that brings the relationship into sharp focus.

#### 68 **Field studies of *Aedes aegypti* control tools in Thailand**

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US military operations in temperate, sub-tropical, and tropical regions face significant risks and impacts from diseases transmitted by mosquitoes. Such diseases as dengue, chikungunya, and yellow fever, transmitted by *Aedes aegypti* mosquitoes, are global threats to military and civilian populations. The Deployed Warfighter Protection Program (DWFP) has evaluated and developed a number of techniques to reduce populations of mosquitoes and the diseases that they transmit. Some of the insecticide spray equipment and insecticides developed under the DWFP program have been approved by the Armed Forces Pest Management Board for use by military preventive medicine units but many have not been evaluated operationally to control *Ae. aegypti* mosquitoes. We field tested 2 pieces of spray equipment and several insecticides in Thailand to evaluate their efficacy in controlling sentinel and naturally occurring populations of *Ae. aegypti* inside houses. We report here the results of equipment performance and efficacy trials conducted during both the hot and rainy seasons of 2013 in villages in Kampheng Phet Province, Thailand. The results of these field trials will drive policy and recommendations by the Armed Forces Pest Management Board's Equipment and Pesticides committees, and are illustrative of translational research supported by the DWFP Program.

#### 69 **Innovative mosquito control concepts**

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Control of vector-borne diseases using insecticides during military deployments is essential to maintain force strength. During a deployment contingency, the amount of equipment and insecticides available is often limited, necessitating compact highly portable equipment that maximizes effectiveness of insecticides for operational deployment. In what appears to be mature science, how does one look at the potential for creativity to make significant improvements to the vector control applications? This presentation will focus on myths of the innovation process and how one can make insightful observations on current vector control technology to make improved equipment and materials. Examples of new ideas that may be useful in controlling insects will be shown, specifically: novel cold fogger, spreading agents, and electrostatic droplets.

#### 70 **Delivery of dsRNA to vector species: successes, challenges, and future directions**

Alden S. Estep, alden.estep@ars.usda.gov, Neil D. Sanscrainte and James J. Becnel

The delivery of double-stranded RNA (dsRNA) constructs to vector species like mosquitoes and sand flies is a critical scientific issue to be overcome in bringing effective RNA interference (RNAi or gene silencing) based pesticides to the field. One proposed delivery method is the introduction of constructs orally. This has been demonstrated to be effective in a number of acarine and insect orders against the organism or host-specific pathogens. Some organisms, like ticks and a number of coleopterans, respond vigorously to orally introduced dsRNA resulting in reduced reproductive output, loss of function, or increased mortality. Effective delivery to dipterans, i.e. mosquitoes and other flies, is more challenging, although there have been some gains. Numerous reports have demonstrated that dsRNA can be introduced into larval mosquitoes by soaking and into adults by feeding on a sugar bait but the mechanism is unclear and the overall effect has

not been robust. Here we review the current methods of dsRNA delivery to vector species and the direction of future DWFP research in this area.

### 71 Functional micro-dispensers (FMDs) for controlled spatial release of insecticides

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Functional micro-dispensers (FMDs) were developed for controlled release of insecticides for optimized spatial coverage, maximized protection with prolonged use and limited chemical exposure. Computational fluid dynamics (CFD) models were utilized for design taking into account the physical properties of the device relative to the repellents used (e.g., sizes of output pores, surface area of evaporation, thermodynamics) at the micrometer and nanometer scales. Two device versions were developed for personal and field use. A number of insecticides were investigated including DEET, transfluthrin, and 1-methylpiperazine. Polymeric devices were fabricated using state-of-the-art SLA 3D printing. Thin film coatings were also utilized to investigate release rates as a function of surface material hydrophobicity. Preliminary *in vivo* studies with human subjects were performed by exposing an adult arm to a cage filled with mosquitoes. Experimental results showed mosquito-bite count reduction of approximately 90% for a prolonged efficacy 3-4 wk relative to no devices. FMDs can deliver known effective spatial insecticides at rates that can guarantee area protection against mosquitoes while minimizing environmental impact.

### 72 Non-toxic insect-resistant textiles for military clothing and equipment

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Current methods used to protect military personnel against vector borne disease rely on the application of insecticidal formulations to soldiers' clothing or ancillary equipment such as bed nets. While effective, these treatments possess some inherent risk due to unknown effects of long-term human exposure to the insecticides used. In addition, it is expected that there will be some transfer of the chemical treatment to the environment due to laundering, use, and disposal. The most serious drawback is the development of resistance to currently available insecticides. Therefore, development of novel, physical methods to deter and prevent insect bites is highly desirable. These alternative materials will mitigate the occurrence of vector resistance to insecticides, and provide an economical and effective alternative to current chemically treated materials. In order to optimize the comfort and safety of these textiles, we used three-dimensional fabric modeling and simulations of insect/fabric interactions to develop a set of prototypes with controlled pore size, pore area, and thickness variability. These prototypes are being evaluated in a novel textile bioassay system, using both *Aedes aegypti* and *Anopheles gambiae* test populations. Preliminary results indicate proof-of-concept. Further evaluation using combinations of materials will be used to create a set of test fabrics that will be evaluated in arm-in-cage studies.

## Advances in Resistance Management in Mosquitoes to Larvicides and Adulticides II

### 73 Global status of resistance to pyrethroids in mosquitoes

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Mosquito control with massive sprayings of insecticides greatly limited mosquito populations and eradicated malaria in a few areas. However, the resistance of mosquitoes to insecticides has become a critical issue that has sparked research projects. Investigators are attempting to elucidate mechanisms of insecticide-resistance development and to develop novel strategies for controlling resistant mosquitoes. Two major mechanisms that are involved in pyrethroid resistance in mosquitoes are: 1) increased metabolic detoxification and 2) decreased sensitivity of target sites. Cytochrome P450s are known to play an important role in detoxifying insecticides in mosquitoes. Transcriptional up-regulation of P450s results in increased P450 proteins and increases P450 activities, which causes the development of insecticide resistance. With the availability of the whole genome sequence for mosquitoes, we are able to characterize the expression profiles of P450s and their functions in insecticide resistant mosquitoes. The voltage-gated sodium channel is the target of pyrethroids. Target site insensitivity of sodium channels in the insect nervous system is known to be of primary importance in the development of resistance to pyrethroid insecticides. Recent studies have found that the co-existence of multiple mutations is an important factor for high levels of pyrethroids resistance in mosquitoes.

### 74 Identification of mutations associated with pyrethroid resistance in mosquitoes

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*Anopheles sinensis*, *Culex pipiens* complex, and *Cx. tritaeniorhynchus* are the primary vectors of the major vector-borne diseases in China. The primary approach of vector control is the application of insecticides. Pyrethroids are currently the most widely used pesticides; their extensive use has led to the development and spread of resistance in the target mosquito populations. The para-type sodium channel in insects is the primary target of pyrethroids. Modifications in the target protein structure cause insensitivity of the insect's nervous system to pyrethroids and result in insecticide resistance. Among these mutations, substitution of leucine to phenylalanine (L to F) in the 6th segment of domain II (IIS6) has been clearly associated with pyrethroid resistance in many insect species, including mosquitoes. In our study, L1014F (codon TTA to TTT) was commonly detected in *An. sinensis*, *Cx. pipiens* complex, and *Cx. tritaeniorhynchus* field populations in China. There was a strong correlation between the prevalence of the L-to-F allelic expression at the genomic level and the

level of resistance against pyrethroids. Besides the L1014F mutation, L1014C substitution (codon TTA to TCA) was also widely detected in *An. sinensis* and *Cx. pipiens* complex. In the present study, a modified allele-specific (AS-PCR) method and a real-time fluorescent quantitative PCR method, both sensitive and specific, were developed and applied as valid tools for detecting the 1014F and 1014S alleles, providing useful information on population genetics of mosquito resistance to pyrethroids in Chinese main vector species.

## 75 Advances in mosquito adulticide resistance management in Taiwan

Err-Lieh Hsu, elhsu@ntu.edu.tw and Hsiu-Hua Pai

There are 2 major dengue vectors in Taiwan, *Aedes aegypti* and *Ae. albopictus*. Insecticide resistance detection is an important component in mosquito abatement program. There are 3 goals for this study: 1) to establish bioassay techniques for *Ae. aegypti* and *Ae. albopictus* for the principal groups of insecticides; 2) to determine the insecticide susceptibilities of the dengue vectors in southern Taiwan; and 3) to evaluate the potential applicability of the resistance mechanism-specific biochemical tests compared with bioassay as a possible surveillance tool for use by vector control programs in Taiwan. Bioassay results indicated that a high level of resistance to all insecticides except fenitrothion was found in *Ae. aegypti* strains from southern Taiwan. All *Ae. albopictus* strains were susceptible to propoxur, permethrin, deltamethrin, cyfluthrin, etofenprox and lamada-cyhalothrin. Synergist assays indicated that the synergist reduced resistance to permethrin in *Ae. aegypti* tested strain. The biochemical assays provide more information about the insect population being analyzed. We have evaluated not only environmental management, but we have also evaluated spraying water diluents of *B. thuringiensis israelensis* and emulsion formulations of pyriproxyfen and diflubenzuron which showed effective alternative for mosquito control. The new formulation may be applied extensively to reach difficult corners. These agents are good auxiliary tools in integrated control of larval mosquitoes.

## 76 An update on resistance to adulticides in Latin America

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Insecticides have been extensively used since the 1940s to control the mosquito vectors of disease and have been a vital component in the fight against arthropod infectious-borne diseases in Latin America. Adulticides are applied as indoor residual spray (IRS), space spray and insecticide-treated nets (ITNs). However, resistance has developed to the 4 classes of insecticides currently recommended for adult vector control and is widespread in populations of the major mosquito vector species from Argentina, Brazil, Mexico, Panama, Venezuela, Colombia, Cuba, El Salvador, Dominican Republic, Puerto Rico, and Belize. Resistance mechanisms have been associated with detoxifying enzymes such as alpha-esterases, beta-esterases, mixed function oxidases and glutathione-S-transferases. Resistance is not evolving through unique new mechanisms; rather, existing mechanisms are being enhanced, and cross-resistance is occurring. Multiresistance is becoming widespread as control programs make sequential use of one chemical class after another. Current control programs in Latin America are largely dependent on synthetic pyrethroids. However, pyrethroid efficacy is now threatened by the rise of target-site resistance (kdr) in several important vectors in multiple locations and spreads at a rapid rate. This raises the question of whether pyrethroid-based insecticides should be replaced with other alternatives to maintain and restore susceptibility to these insecticides in Latin America.

## Disease Vector II

### 77 Seasonality of West Nile virus transmission to sentinel chickens in the absence of human cases

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Florida has not experienced major outbreaks of human West Nile cases, yet annually recurring, sometimes locally intense, transmission to sentinel chickens is observed. West Nile virus (WNV) was first detected in Indian River Co., FL, in sentinel chickens, and 195 sentinel WNV seroconversions have been documented since the initial detection in 2001. Intense transmission to sentinels led to issuance of local mosquito-borne illness advisories or alerts in 4 yr, providing meaningful "early warning" of risk to man. To date no human West Nile cases, no wild avian die-offs and only 2 equine cases have been documented in Indian River Co. Annual seasonality of transmission has shown considerable variation, probably as a result of the bionomics of the region's predominant vector, *Culex nigripalpus*. *Culex nigripalpus* is fundamentally a floodwater mosquito, vagile and extremely abundant. Patterns of West Nile activity in Florida may differ from those seen elsewhere in the US, in part as a result of regional differences in vector species and climate.

### 78 Mechanism of action of N,N-diethyl-meta-toluamide (DEET) neurotoxicity

Daniel Swale, dswale@gmail.com and Jeffrey R. Bloomquist

The insect repellent DEET (N,N-diethyl-3-methylbenzamide) is used more often than any other mosquito repellent. Although it has been in use for 60 yr, the mechanism of repellency action and the neurotoxicity have eluded scientists. Recent studies have suggested that DEET is an anticholinesterase and that this action may contribute to its repellent effects in insects and cause risk of toxicity in exposed humans. We have confirmed that DEET is lethal to mosquitoes ( $LD_{50} = 2-4 \mu\text{g/insect}$ ) but observe that DEET is a poor anticholinesterase in mosquitoes (<10% inhibition at 10 mM). Neurophysiological recordings show DEET to possess neuroexcitatory effects to the house fly CNS at micromolar concentrations. Phentolamine, an established octopaminergic antagonist, blocked the CNS neuroexcitation of DEET and

octopamine but was ineffective against propoxur. Additionally, DEET was shown to activate the fire fly light organ through an agonist-like action, a system documented to use octopamine as the primary neurotransmitter. Lastly, DEET was found to increase calcium mobilization at the octopamine receptor in Sf21 cells. These 3 findings suggest DEET is targeting octopaminergic synapses and not acetylcholinesterase to induce neuroexcitation while indicating that anticholinesterase effects do not underlie toxicity or repellency.

**79 Comparison of trapping methods for adult *Aedes albopictus* and *Aedes aegypti* mosquitoes in New Orleans, Louisiana**

Sarah Michaels, smichael@tulane.edu, Andrew Ruiz, Samuel Jameson, Justin K. Davis, Claudia Riegel and Dawn M. Wesson

Previous studies have described relative distributions of *Aedes albopictus* and *Ae. aegypti* in New Orleans, LA. These species are efficient urban container ovipositors, aggressive pests, and important arbovirus vectors. The New Orleans Mosquito and Termite Control Board and Tulane University monitored trap locations throughout the city weekly from May - October. Eggs were collected by ovitrap and reared for species identification. Paired dry-ice baited CDC light traps (CDC) and BG-Lure<sup>®</sup> and octenol lure-baited BG Sentinel<sup>®</sup> traps (BG) were set overnight and their positions were rotated each week. Ovitrap were used as a representation of the relative abundance of each species and adult traps were evaluated for preferential species collection. Initial analysis of adult collection data indicated that in an environment where the 2 species co-occur, there was a preference for the BG to collect *Ae. albopictus* while the CDC trap was equally likely to collect either species. Understanding the inherent bias of different adult mosquito collection traps is necessary if the resulting data are to be used to monitor for risk of mosquito-borne pathogens, and to target control measures.

**80 Genetic relationships of the first isolate of EEEV in the state of Vermont**

John-Paul Mutebi, Grv0@cdc.gov, Alan C. Graham, Erica Berl, Kali Saxton-Shaw, Jeremy Ledermann and Ann Powers

The first outbreak of EEEV in Vermont was reported on an emu farm in Rutland County in 2011. An isolate was obtained from a brain of an emu and phylogenetic analysis showed close relationships with strains from Virginia, Florida, Connecticut, and New York isolated between 2003 and 2005. This shows that the EEEV strain isolated in Vermont in 2011 evolved from strains commonly circulating in eastern US. Furthermore, this observation suggests that Vermont is part of the EEEV activity range and that the outbreak was not as result of a recent expansion

**81 Searching for the AChE mutation in *Anopheles albimanus* from the coastal plain of Chiapas, Mexico**

R. Patricia Penilla, penilla@insp.mx, Elizabeth Fierro Rabanales, Americo D. Rodriguez, Macario M. Fierro, Mylene Weill, Arnaud Berthomieu, Karla Saavedra-Rodriguez, Francisco Solis, Alma D. Lopez and William Black

Previous studies made on the malaria vector from the coastal plain of Chiapas had shown low resistance levels to carbamates and organophosphates, suggesting by biochemical assays the presence of an AChE mutation (Penilla et al, 1998). Nowadays, in the Mesoamerican malaria pre-elimination context and considering the widespread pyrethroid resistance in many places, it is necessary to establish baseline studies regarding the presence of 1 of the major carbamate and organophosphate insecticides target site resistance mechanisms. Recent results showed no resistance to a carbamate (propoxur) insecticide using the WHO tests at the discriminant concentration. In order to obtain mosquito survivors to these insecticides, we exposed them only during 15 min to the discriminating dosages suggested by WHO, in an attempt to detect heterozygous. DNA was extracted from those dead and alive mosquitoes and sequenced, and PCR products tested by the diagnostic method suggested by Weill et al 2004. So far, AChE mutation has been not detected in 3 localities from Chiapas (Buena Vista, Pijijiapan; Emiliano Zapata, Mazatan; and Brisas del Mar, Suchiate), except for 1 single heterozygous from Buena Vista. Possible changes in the land use and consequently insecticide usage in this region could have been favoring a reversion of the previous AChE levels detected. Further studies increasing the sample size are being conducted.

**82 Initiation of *Anopheles gambiae* surveillance in Liberia by LIBR**

Mawolo N. Kpawor, mkpawor@yahoo.com, Joseph W. Diclaro and Fatorma K. Bolay

The West Africa country of Liberia has a long history of scientific research conducted by the Liberia Institute for Biomedical Research (LIBR). However, these research activities were interrupted during a 14-yr civil war that destroyed Liberia's infrastructure. With collaboration with the Naval Medical Research Unit No. 3 and funding from Global Emerging Infections Surveillance & Response System Operations (GEIS), LIBR has been able to start rebuilding capacity and start recontributing to the scientific community with the vector surveillance, malaria detection, and GIS mapping of *Anopheles gambiae* in an effort to focus control measures.

**83 Epidemiology of West Nile virus in New York City: analysis of mosquito data, 2002- 2013**

Waheed I. Bajwa, wbajwa@health.nyc.gov and Marcia O'Connor

West Nile virus (WNV) first appeared in New York City in August of 1999. Since then, the virus has found a permanent home in New York City (NYC) and spread to every state in the continental U.S. The virus shows up every year in the hottest months of July and August in NYC. In the last 15 years, the city has experienced several outbreaks; the most

significant was in 1999 when the infections resulted in 59 human cases, including 7 deaths. There was also, higher than normal activity in 2002, 2003, 2010, and 2012. Since 1999, the Department of Health & Mental Hygiene has collected a significant amount of data on this virus, its vectors, and their role in the transmission of the virus in NYC. Recently we have analyzed these data; in this paper, we will discuss the role of biological and ecological factors associated with the outbreaks of the virus in NYC.

**84 Contribution of social mobilization for the monitoring of *Aedes albopictus* in France, a new tool: iMoustique**

Sébastien Chouin, sebastien.chouin@eidatlantique.eu, Michel Marjolet, Jérôme de Maupeou and Paul Lechuga

Since the first interception in France of *Aedes albopictus* in 1999 by EID Atlantique, the French Ministry of Health has set up a health plan aimed at the early detection of this species in the unaffected territories to prevent vector-borne diseases in France. Until 1999, entomological surveillance of invasive mosquitoes was essentially based on a network of strategically located traps. From 2011 to 2012, EID Atlantique publicized a reporting procedure for the tiger mosquito allowing people with a mobile phone to connect directly on the web report form. In 2013, EID Atlantique has developed the first application on mosquitoes (iMoustique®) which directly transmits a picture from the phone. Since 2012, more than 1,000 reports have been received which permitted us to confirm establishment of *Ae. albopictus* in 6 new towns. In comparison to 2012, the proportion of reported mosquitoes was more than 70% in 2013. Considering the current spread of *Ae. albopictus* in France, entomological monitoring by traditional trapping methods reached certain limits. The objective of this first application on mosquitoes is to enhance citizen watch, which has already demonstrated its effectiveness. This new tool allows us to survey exotic mosquito species and contributes to a better knowledge of local species. This educational approach strengthens the entomological monitoring network and enhances vector risk awareness.

**85 Tick and tick-borne disease surveillance in Washington State**

Elizabeth Dykstra, elizabeth.dykstra@doh.wa.gov, Jo Marie Brauner, Ashlee-Rose Ferguson and Michelle Jodziewicz

Washington is home to several tick-borne diseases, including Lyme disease, tick-borne relapsing fever, Rocky Mountain spotted fever, anaplasmosis, tularemia, and babesiosis. Each year a small number of endemically acquired tick-borne disease cases are reported to the state health department. In an effort to better understand the current status of the state's tick populations and associated pathogens, for the past 3 yr the Washington State Department of Health has conducted surveillance for ticks and tick-borne pathogens across the state. Over 4,800 ticks representing 5 genera and 12 species have been collected and identified, including *Ixodes pacificus*, *Ix. angustus*, *Ix. spinipalpis*, *Dermacentor andersoni*, and *D. variabilis*. A total of 737 *Ix. pacificus*, *Ix. angustus*, and *Ix. spinipalpis* ticks were tested for *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia* species; 280 *D. andersoni* and *D. variabilis* ticks were tested for *Rickettsia rickettsii*. Twelve (1.6%) ticks tested positive for *B. burgdorferi* and 5 (0.7%) were positive for *A. phagocytophilum*. None of the *Dermacentor* ticks tested positive for *R. rickettsii*; however 4 species of non-pathogenic *Rickettsia* were detected in 29 (21%) of 140 *Dermacentor* ticks screened for *Rickettsia* species. These findings indicate that both *B. burgdorferi* and *A. phagocytophilum* are present in Washington's *Ixodes* tick populations at low levels.

## Latin America Symposium II

**86 Vector borne diseases in the Wayúu ethnic group of Marbacella, La Guajira - Colombia**

Edith N. Gomez Melendro, natalia.gomez@ecosaludetvcolombia.org, Laura Castro Díaz, Sofía Díaz, Helena Brochero and Catalina Gonzalez

Indigenous groups in Colombia represent 3.43% of the total population. They are located in areas with socio-ecological areas where vector-borne diseases are an important public health concern. We present the ecological, biological, and social aspects related to insect vectors for the Wayúu ethnic group of Marbacella, Riohacha, La Guajira, Colombia. Near human dwellings, we collected triatomine and *Anopheles* species. Larval sites for malaria and dengue vectors were characterized and light traps were used to capture vectors of trypanosomiasis and malaria. For the ecological and anthropological component, geo-referencing routes, KAP surveys, semi-structured interviews, and participatory workshops were conducted. *Anopheles* spp. were only associated with the rainy season, although their oviposition sites remain active throughout the year. *Triatoma maculata* was encountered near human habitations, and this was the first report of this species for the municipality. The local people call the triatomines “Ishisü” and the mosquitoes “Mei” and consider mosquitoes as a factor of financial risk because they affect their livestock. The vegetation is typical of tropical dry forests with predominance of legumes and Cactaceae. This baseline study seeks, with the community, to define the community integral strategies for control and prevention of vector-transmitted diseases from an ecohealth framework.

**87 Ecohealth approach to determinate vector-borne diseases in Bari- Karikachaboquira indigenous group, Tibú, Norte de Santander, Colombia**

Angelica M. Torres Naranjo, angelica.torres@ecosaludetvcolombia.org, Andres Felipe Santo Domingo, Sofía Díaz, Johanna Yañéz, Eulides Pabón, Helena Brochero and Catalina Gonzalez

We describe the entomological and eco-social baseline to define prevention and control strategies from an ecohealth approach among the Bari-Karikachaboquira indigenous group located in the municipality of Tibu, Norte de Santander, Colombia. Triatomine and *Anopheles* spp. were captured in association with dwellings, larval sites for malaria and dengue



vectors were characterized, and light traps were used to capture leishmaniasis and malaria vectors. *Anopheles nuneztovari* and *An. rangeli* were the most abundant malaria vectors. *Rhodnius prolixus* is recognized by the community as a vector of Chagas disease and is called “Shidrú.” Bari people did not recognize leishmaniasis vectors, but ecological conditions are adequate for them to be present. Vegetation belongs to the tropical wet zonobiome from Catatumbo including tree palm, cocoa, and banana crops where mosquitoes were found resting. The Bari community perceives malaria and Chagas as important diseases; autochthonous prevention and control strategies are being developed. Ecohealth strategies among indigenous communities must integrate other disease priorities such as tuberculosis and intestinal parasitism along with vector-borne diseases to develop sustainable and culturally sound interventions.

**88 Ecological calendar as a methodological tool for the design of dynamic interventions for the prevention, surveillance and control of vector-borne diseases in two indigenous communities in Colombia**

Felipe SantoDomingo, felipe.santodomingo@ecosaludetvcolombia.org, Laura Castro-Díaz, Helena Brochero and Catalina Gonzalez

Local ecological knowledge is recognized as one of the most important sources of information to determine social and ecological annual dynamics. Every year communities experience rainy seasons, drought, abundance or lack of food, and disease outbreaks, among others. The present study presents the ecological calendar for Karikachaboquira, a Barí ethnic group community, and in Marbacella of the Wayúu ethnic group. Information was collected through seasonal graphics, semi-structured interviews, participatory observation, and georeferencing routes. Collected information was triangulated in order to validate and complement the results. For each community, we obtained an ecological calendar built from local knowledge, which shows socio-ecological dynamics associated with vector-borne diseases. We observed that local dynamics change depending on environmental conditions and that these in turn determine the presence or absence of insect vectors, enhancing or decreasing the risk of transmission. For example, the vectors of malaria are more associated with rainy season because the larval habitats of *Anopheles* spp. increase compared to the dry season. Local people who do their hunting and fishing activities change their work schedules because of environmental conditions, increasing the risk of malaria transmission. Through an ecological calendar, socio-ecological dynamics are represented graphically providing evidence to develop sound prevention strategies.

**89 Fishing annual dynamics and its relation to malaria transmission among the Wayúu indigenous group in Marbacella and El Horno, La Guajira-Colombia**

Laura Castro-Díaz, laura.castro@ecosaludetvcolombia.org, Daniel Garzón and Catalina Gonzalez

Community knowledge is vital to understand the ecological and cultural dynamics that occur in local environments. This paper aims to identify the annual fishing dynamics among the Wayúu people in Marbacella and El Horno in Colombia and its relation to malaria transmission in order to define intervention strategies from an ecohealth framework. We conducted a participatory workshop using a seasonal graphic, 7 semi-structured interviews, geo-referencing routes and ethnographic observation. Variables such as climatic seasons, socioeconomic activities, presence of *Anopheles* spp., and cultural events that occur throughout the year were analyzed. The annual socioeconomic dynamics in Marbacella and El Horno change depending on weather conditions. During the rainy season, the inhabitants of this community reported changes in fishing schedules, activities, zones, and practices. Local fishermen start their work activities between 1:00 and 2:00 a.m. and end at 5:00 a.m. To perform their tasks, fishermen have to walk from their homes to the beach through vegetation cover in which oviposition sites of *Anopheles* spp. are present, increasing the risk of malaria transmission as a result of increased vector biting rates during this time period. There is a direct relationship between rainy seasons, the presence of the insect vectors, malaria cases, and changes of socio-economic activities such as fishing practices.

**90 The armed conflict actors as enhancers of vector-borne diseases in Catatumbo, Colombia**

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The Catatumbo is currently an area of national interest in Colombia as an epicenter of political, socio-economic and environmental conflicts because of its geographical location and rich natural resources. The Barí indigenous people of Karikachaboquira, who inhabit this territory, have created belief systems and have naturalized the dynamics associated with the conflict that they experience in their lives. These belief systems provide causal explanations for the presence of vector-borne diseases. The aim of this study is to describe and analyze these social imaginaries. Information was gathered through semi-structured interviews, focus groups, informal discussions, social mapping, and historical graphics to learn about the ecological and social changes that have taken place in different time-frames. Community participants reported that there are insects that carry diseases. From a local perspective, these insects “suck” blood, a behavior which is associated with evil beings, and linked directly with the different actors in the local armed conflict. These actors travel actively in the area walking through different landscape units such as plantain, cassava and cocoa cultivation, floodplain forest, upland forest, pastures and stubble. Furthermore, the area is influenced by coca plantations and oil palm; both crops enhance political socioeconomic and environmental conflict and are associated to vector breeding sites.

**91 Sociocultural dynamics of therapeutic itineraries in two indigenous groups in Colombia**

Daniel A. Garzon-Moreno, daniel.garzon@ecosaludetvcolombia.org, Elkin Vallejo and Catalina Gonzalez

Analyses of local therapeutic itineraries characterize routes and actors involved in healing practices. The aim of the study was to describe from an ecohealth framework the processes and dynamics of the therapeutic itineraries among 2 indigenous groups in Colombia: the Bari group of Karikachaboquira and the Wayúu from Marbacella and El Horno. Semi-structured interviews, focus groups and knowledge dialogues were conducted with the community, medical doctors, health providers and traditional knowledge leaders. This was done to identify primary treatments for diseases and what were the medical and technical criteria and requirements needed, before and after visiting a traditional doctor, and/or health promoter and the hospital. For the Bari, dengue treatment is provided from a western medicine precept; the first step of the healing process is going to the community health promoter and concludes with the completed healing process in a municipal or departmental hospital. In contrast, for the Wayúu people, the healing process associated with malaria is linked to members of the Departmental Ministry of Health who provide diagnosis and treatment, because of its proximity to the city of Riohacha and in the absence of traditional healers in the area. Therapeutic itineraries for indigenous communities vary according to processes of health syncretism and geographical barriers.

**92 Alpha and beta diversity of phlebotomine sand fly assemblage (Diptera: Psychodidae: Phlebotominae) from Calakmul, Mexico**

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It is a well-established fact that cutaneous leishmaniasis is endemic in southern Mexico and it is caused by *Leishmania mexicana*. Several sand fly species such as *Bichromomyia olmeca olmeca* [= *Lu. olmeca olmeca* (Vargas y Díaz-Nájera)] as well as *Lutzomyia (Tricholateralis) cruciata* [= *Lutzomyia cruciata* (Coquillett)], *Psychodopygus panamensis* [= *Lutzomyia panamensis* (Shannon)], and *Psathyromyia (Psathyromyia) shannoni* [= *Lutzomyia shannoni* (Dyar)] may act as vectors. As a result of deforestation in the region, transmission cycles may vary from time to time and from site to site. Even though Calakmul, Campeche, is an endemic county for leishmaniasis, to this date no study has focused on the diversity of the assemblage Phlebotominae to compare the presence and abundance of sand fly species among several locations. Seven locations in Calakmul were sampled from 2001 to 2007, and species richness and abundances for each site were recorded. Diversity indices were calculated and compared between locations. A total of 17,405 specimens of 17 species were collected. The highest richness was in locations Once de Mayo and Dos Naciones, while Q-statistic was highest in Dos Laguna Sur and La Guadalupe. and the highest dominance was observed in Unión 20 de Junio and Arroyo Negro. Beta diversity (similarity) revealed that in most instances sand fly species were similar from site to site; although there were differences between sites, they did not correlate with geographical distances. C-score analysis of presence/absence data showed no significant differences among communities, therefore the pattern of species co-occurrence is random.

**93 The *Ochlerotatus triseriatus* group (Diptera: Culicidae) in Mexico**

Aldo Ortega Morales, agrortega@hotmail.com, Felix Ordoñez, Adelfo Sanchez, Teresa Valdes, Javier Sanchez and Thomas Zavortink

The *triseriatus* group includes *Ochlerotatus brelandi* which occurs in southwestern Texas and northeastern and central Mexico. *Oc. hendersoni* occurs in southern Canada and United States except the western and southwestern regions; *Oc. triseriatus* occurs in southeastern Canada, the eastern United States and northeastern and central Mexico; and *Oc. zoosophus* occurs in the south central United States, northeastern and southern Mexico. In Mexico, species of the *triseriatus* group may occur together. In Nuevo Leon, *Oc. triseriatus* and *Oc. zoosophus* were collected in the same tree hole. In Tamaulipas, *Oc. zoosophus* were collected in a discarded tire, while *Oc. brelandi* was collected biting/landing, and *Oc. brelandi* and *Oc. triseriatus* were collected biting/landing. In Queretaro, *Oc. brelandi* and *Oc. triseriatus* were collecting biting/landing. *Ochlerotatus triseriatus* is the main vector of La Crosse virus (LACV), which affects the central nervous system and can be severe and deadly, especially among young children. LACV is not surveyed in Mexico, so its prevalence and incidence are unknown. The United States has had reports of LACV, and considering the geographical closeness with northern México, entomological surveys for *Oc. triseriatus* and conducting epidemiological surveys for LACV are strongly recommended by Mexican health authorities in states where this species has been reported.

**U.S. Military Vector Control Research and Development: Ten Years of Translational Research in the Deployed Warfighter Protection (DWFP) Program II****94 AFPMB-USDA European field facility for vector control research, 2011-2013**

Alexandra Chaskopoulou, andahask@ufl.edu, Javid Kashefi, Roberto Pereira and Philip Koehler

In 2011, a vector control research program was initiated at the USDA Agricultural Research Service (ARS) European Biological Control Laboratory's field station at Thessaloniki, Greece. The work produced by this program is a joint effort of ARS and University of Florida scientists under the financial support of the Deployed Warfighter Protection Program (DWFP). DWFP is an initiative from the US Department of Defense, managed by the Armed Forces Pest Management Board (AFPMB). The main objective of this research endeavor is to optimize currently available vector management

practices in the field, by integrating different control methodologies to achieve maximum and long lasting results. The main target insects studied in the field include, but are not limited to, *Phlebotomus* sand flies (*Phlebotomus perfliewi*, *P. tobbi*, *P. simici*, and *P. papatasi*), major mosquito vectors in the Mediterranean basin (*Anopheles sacharovi* and *Culex pipiens*) and the common house fly, *Musca domestica*. Field sites with high levels of pest activity have been identified and the behavior and seasonal activity of each target pest was studied on site prior to any treatments. Treatments included: a) ULV applications of deltamethrin-based products against mosquitoes and sand flies, b) attractive targeted sugar bait applications with boric acid against mosquitoes and sand flies, and c) integration of the 2 methodologies against mosquitoes and sand flies.

### 94a Field application of toxic sugar baits (TSB) to control sand flies

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Sand flies (Diptera: Psychodidae: Phlebotominae) are the source of nuisance biting and transmit leishmaniasis, sand fly fever and bartonellosis. The difficulty of controlling sand flies by conventional methods and their public health importance to deployed forces and civilians necessitate the development of alternative approaches. One promising alternative for sand fly control is to use toxic sugar baits (TSB) containing low doses of neonicotinoids or other insecticides. The feasibility of using this method against sand fly on a wide scale necessitates optimization and standardization to achieve satisfactory levels of control in diverse situations with minimal harm to the environment and non-target organisms. The results of a series of field experiments conducted in Israel in 2012 and 2013 indicate that a high proportion of sand flies feed on sugar solutions mixed with food dye sprayed on shade-nets placed vertically. After the spraying of TSB containing dinotefuran (0.3gr ai/m<sup>2</sup>) on net-barriers, no marked sand flies reached traps placed at a distance of 2.5 m from the barriers for at least 3 wk, demonstrating a fast and residual activity of the insecticide. Further experiments are underway to improve the persistence of TSB in the field and the percentage of exposure of sand flies to the baits.

### 95 Design and development of novel insecticides with minimal non-target risk

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Two sub-projects have been initiated under DWFP support since 2009. A novel hormone-induced cell transformation technique (patent pending) was developed in order to facilitate high throughput screening and insecticide discovery, without the requirement of genetic engineering. This method induces expression of ion channels (e.g., potassium channels) that display the expected responses to established pharmacological agents and are appropriate for chemical insecticide screening. Other work evaluated a series of essential oils as candidate synergists of carbamate and pyrethroid insecticides in mosquito larvae and adults. This study found reductions in metabolic enzyme activity that could explain the modest synergism observed. No effects could be ascribed to target site interactions.

### 96 Green chemistry approach to vector control

Joel R. Coats, jcoats@iastate.edu, Aaron D. Gross, Edmund J. Norris and Lyric C. Bartholomay

Infectious diseases spread by mosquitoes are significant problems in many locations throughout the world. The yellow fever mosquito *Aedes aegypti* is the primary vector of dengue virus, a rapidly spreading mosquito-borne virus. *Anopheles gambiae* is a vector of the malarial *Plasmodium* parasites. Chemical control of mosquitoes has been hampered by: 1) increasing insecticide resistance, and 2) increased public concern about the safety of chemical insecticides. Use of chemical insecticides with synergists, like piperonyl butoxide (PBO), has been partially effective at overcoming resistance and reducing amounts of insecticides used. Terpenoids in plant essential oils have been shown to be mildly effective in killing mosquitoes. In addition, essential oils and their terpenoids can enhance the potency of natural pyrethrins. In an effort to identify plant essential oils that could be utilized to enhance the activity of synthetic pyrethroids, 35 plant essential oils, from various plant families, were topically applied to adult female *Ae. aegypti* and *An. gambiae*. In addition, mixtures of plant essential oils and a commercially available permethrin formulation were analyzed for enhanced efficacy and compared to PBO. Plant essential oils are less toxic than conventional synthetic pyrethroids; however, some have similar or better efficacy as enhancers of pyrethroid efficacy compared to PBO.

### 97 First decade of progress seeking new pesticides for Deployed War-Fighter Protection

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To replenish the range of pesticides available for vector control and personnel protection, the Armed Forces Pest Management Board secured funding (\$5M/year since 2004) to support research by the USDA-ARS and other collaborators. Priority is given to discovering insecticides with novel modes of action, particularly adulticides with fast knockdown and low hazard for humans and the environment. Several new classes of insecticide have been designed and synthesized, optimized by QSAR/QSPR modeling and are now in development. Most exciting was the patenting of dsRNA targeting vital genes in mosquitoes, leading to the transformational science of specific RNAi pesticides. Insect repellent chemicals of many types have been isolated from plants and synthesized, some more potent and longer-lasting than DEET. Their modes of action are being characterized to provide the most effective, safe combinations of synergistic repellents.

## Latin America Symposium III

### 99 Molecular cloning and characterization of the circadian clock timeless gene in *Culex quinquefasciatus* Say (Diptera: Culicidae)

Iram P. Rodriguez-Sanchez, iramrodriguez@gmail.com, Olga K. Villanueva-Segura, Rafael González-Alvarez, Adriana E. Flores-Suarez, Laura E. Martínez-de-Villarreal, Hugo A. Barrera-Saldaña and Gustavo Ponce-García

In prokaryotes and eukaryotes, the gene codifying for TIM protein has an important role in the circadian clock that regulates rhythmic feature of: biochemical, physiological, and behavioral processes. TIM messenger RNA amplification was performed by RT-PCR from *Culex quinquefasciatus* larvae (southern house mosquito) collected from 3 locations at the State of Nuevo Leon, northeastern Mexico. The amplified products were cloned and sequenced; the revealed cDNA was equivalent sequences from other species of mosquitoes. A 3062 bp amplified product was synthesized by PCR, from 3 overlapping cDNA fragment to complete CDS (2991 bp) of TIM. Its encoding ORF for of 996 amino acid residues, turned up to be dramatically smaller than the orthologous in other mosquitoes analyzed. Serial deletions in the core of the sequence were visualized in *Culex quinquefasciatus* TIM transcript. No other amplification products were visualized, and thus we discard the possibility of alternative spliced mRNA species. As TIM is a widely conserved gene in eukaryotes, and maintaining *Culex quinquefasciatus* colonies is relatively easy, determining of the sequence of the TIM gene in the mosquito provides the possibility of being used as a model of experimentation for of the circadian clock.

### 100 Semiochemical manifestations of essential oils from *Cymbopogon flexuosus* against the dengue vector *Aedes aegypti* (Diptera: Culicidae)

Sharon S. Vera, sharonsmithvera@hotmail.com, Ruth M. Castillo, Elena E. Stashenko and Jonny E. Duque

Essential oils of plants are means pest control understudied. In this work, the semiochemical manifestations of the essential oil of *Cymbopogon flexuosus* (Poaceae) on *Aedes aegypti* (L) were studied. Bioassays were carried out at different *Ae. aegypti* Rockefeller development stages. Larvicidal activity was determined by lethal concentrations (LC<sub>50</sub> and LC<sub>95</sub>) using Probit analysis. At concentrations of 250, 310 and 390 ppm, pupicidal activity was estimated. Adulticidal activity was estimated by impregnation of glass bottles with essential oil at 30, 300, and 1000 ppm, the adulticidal activity was evaluated. Mosquitoes with a physiological age between 2 and 5 days after they were emerged from the pupae were selected. Mortality lectures were carried out at 24 h. Bioassays followed the guidelines of the World Health Association with slight modifications. Results indicate that the essential oil of *C. flexuosus* presents larvicidal activity LC<sub>50</sub>=17.16ppm (I.C=13.78-21.37) and LC<sub>95</sub>=49.9ppm (I.C=30.92-80.66). The pupicidal activity was at 250 ppm=6.6±0.5%, 310 ppm=13.3±0.5%, 390 ppm=20±1%. The adulticidal activity was at 30 ppm=40±4.2%, 300 ppm=62.5±12% y 1,000 ppm=96.26±0.7%. We conclude that the essential oil from *C. flexuosus* presents insecticidal activity in all the evaluated stages of *Ae. aegypti*.

### 101 Essential oil of *Cymbopogon flexuosus* (Poaceae): analysis in silico, repellency in the oviposition and protection against the biting dengue vector *Aedes aegypti* (Diptera: Culicidae)

Ruth M. Castillo, rcastillo@jdc.edu.co, Sharon S. Vera, Aurora L. Carreño, Elena E. Stashenko and Jonny E. Duque

The biodisponibility and security of major compounds (MC) presented in the essential oil (EO) from *Cymbopogon flexuosus* -Citral and Geranyl acetate- were analyzed. The humans protection by repellency against the biting of *Aedes aegypti* (L) using the EO was also determined. The oviposition response of *Ae. aegypti* (L) to the essential oil was evaluated. The ADMET parameters, which are used to predict the utility of the MC, were estimated in silico. The protection against biting was evaluated with unfed female mosquitoes (N=20) newly emerged (2-5 days), by exposition of the forearm of a volunteer at 1,000 ppm of EO. The oviposition repellency was determined in containers with EO (at 5, 50 and 200 ppm) using female mosquitoes. The oviposition activity index (OAI) and the protection percentage (PP) were calculated. The ADMET parameters for the MC presented acceptable values and a low risk to mammalian. A protection percentage of 58.33±1.5% during 1h of exposition was obtained. EO of *C. flexuosus* is repellent to oviposition (OAI= -1) with an EP% = ≥ 80%. We conclude that the EO of *C. flexuosus* is safe, acts as repellent for oviposition, and can also protect against *Ae. aegypti* biting.

## New Product Trials

### 102 How do we replace Scourge for control of the southern house mosquito, *Culex quinquefasciatus*?

Scott Willis, swillis@cppj.net, Scott Harrington, Jill Hightower and John Rowland

Historically Calcasieu Parish has used Scourge ground ULV applications against field mosquito populations of *Culex quinquefasciatus*. In order to find a Scourge replacement, a new mosquito adulticide was evaluated against field-collected *Culex quinquefasciatus*. This presentation will discuss application rates and results from this research.

### 103 MetaLarv S-PT® field efficacy trial in Gem County Mosquito Abatement District

Jason R. Kinley, director@gcmad.org

MetaLarv S-PT® residual larvicide was applied in several flood-irrigated hay fields and pasturelands in the Gem County Mosquito Abatement District. Product efficacy over time and through several dry down and re-wet cycles was analyzed. Future use and expectations in the Gem County Mosquito Abatement District will be presented.

**104 Field efficacy trials supporting registration of a new mosquito adulticide for use against flies of importance in public health**

Jessica Lawrence, jessicalawrence@eurofins.com, Kurt P. Vandock, Jing Zhai, Paleta Rekowsky and Byron L. Reid

Mosquito adulticides are often used to control other important flying pests, such as black flies or stable flies. To support EPA registration for this use pattern, field efficacy studies were conducted in North Carolina against 5 fly species. This presentation will discuss methods and results from this research.

**105 Field efficacy trials supporting registration of a new active ingredient for use as a mosquito adulticide**

Kurt P. Vandock, kurt.vandock@bayer.com, Jing Zhai, Paleta Rekowsky and Byron L. Reid

In past decades, there have been very few new active ingredients registered for use as mosquito adulticides. We will present results from 4 geographically diverse field studies documenting the efficacy of a new adulticide formulation against more than 10 species at application rates as low as 0.0005 lb AI per acre.

**106 A new active ingredient for ULV application to control adult mosquitoes in the United States**

Gordon Morrison, gordon.morrison@bayer.com, Kurt P. Vandock, Britt Baker and Byron L. Reid

Bayer has developed a new active ingredient for use in the control of adult mosquitoes by area-wide, ULV applications. Submission was made to the EPA in September 2013, and commercial introduction will begin in 2015. This presentation will: review the history of the development of the new insecticide, profile important features of the active ingredient and formulation, plus outline the labeling submitted to the Agency.

**107 Comparative efficacy of the biopesticide ethyl butylacetylaminopropionate (IR3535) repellent to mosquitoes, ticks, fleas and flies**

Howard A. Epstein, howard.epstein@emdillipore.com and Anja Martina Bohlman

IR3535 repellent efficacy at concentrations 10%-30% was compared to a positive control DEET. Ethanol solution, cream and pump spray vehicles were evaluated in arm-in-cage and field tests conducted in globally diverse regions using *Aedes aegypti*, *Culex* sp., *Ae. albopictus*, *Anopheles* sp. and flies; *Simulium* sp., *Stomoxys calcitrans*, deer tick *Ixodes scapularis* and cat flea *Ctenocephalides felis*. Arm-in-cage studies had equivalent repellency for *Ae. aegypti* for IR3535 and DEET, mean protection time varied (2 to 9.8 h). The vehicle used was as important as repellent concentration. For *Ae. albopictus*, repellency protection of field tests ranged from 4 to 8 h, efficacy of IR3535 comparable to DEET. Another study using 2.5-5% active in an aerosol vehicle gave protection of 5-6 h; IR3535 and DEET were comparable. For *Anopheles* sp. field testing using a lotion vehicle at 15% and 25% active in ethanol resulted in mean protection times of 5-6 h. Mean protection times for cage studies varied depending upon the species of *Anopheles*. IR3535 and DEET protection times were comparable except in a study using *An. maculates*. An ethanol vehicle at 25% active resulted in DEET providing better repellency protection for the first hour, at the second hour DEET and IR3535 were comparable. A deer tick study with *Ix. scapularis* compared a lotion vehicle (10% IR3535) to a pump spray (20% IR3535) and aerosol (20% IR3535) had protection times of 9, 12 and 11 h, respectively

**Vegetation Management and ATSB for Biting Fly Control I**

**108 Overview of vegetation and mosquito relationship**

Rui-De Xue, xueamcd@bellsouth.net

Vegetation is a part of the mosquito ecosystem. All stages of mosquitoes are directly and indirectly associated to vegetation. Several species of mosquito larvae attach to aquatic plant roots for survival and many plants may provide food/water resources, resting, and hiding places for adult mosquitoes, especially males. Therefore, vegetation management is directly and indirectly impacting mosquito population.

**109 Plant tissue, flowering and non-flowering vegetation impacts on mosquito survival**

Whitney Qualls, quallsamcd@bellsouth.net, Ali Fulcher, Rui-De Xue, John Beier, Jodi M. Scott and Gunter Muller

The summary of work evaluating the survival of important vector species by feeding upon plants from their natural habitat in Florida is presented. Plants non-flowering during the study period observed in the laboratory are redbay (*Persea borbonia*), cherry laurel (*Prunus caroliniana*), muhly grass (*Muhlenbergia capillaris*), and coontie (*Zamia pumila*). Plants flowering during the study periods evaluated are elephantsfoot (*Elephantopus elatus*), blanketflower (*Gaillardia pulchella*), spotted beebalm (*Monarda punctata*), beach sunflower (*Helianthus debilis*), firebush (*Hamelia patens*), American beautyberry (*Callicarpa americana*) and false indigo bush (*Amorpha fruticosa*). Throughout the different evaluations mosquito plant interactions have been documented influencing mosquito survival. Mean survival time for *Aedes albopictus* was statistically significant between flowering and non-flowering plants on average. Overall, our findings highlight the complexity and importance of mosquito-plant relationships and how these relationships should be further studied to develop control strategies targeting the necessary sugar feeding and plant tissue feeding behavior of mosquitoes.

**110 Vegetation as a sugar resource**

Woodbridge Foster, foster.3@osu.edu

Almost universally, adult biting Diptera derive sugar from plants. Therefore, they may be considered mixed-diet foragers that balance their needs from 2 very different kinds of food: plant sugar and vertebrate blood. Among sand flies, biting midges, mosquitoes, and tabanids, the males feed exclusively on sugar. Even those synanthropic species whose females can survive and reproduce on human blood alone commonly sugar-feed and apparently derive fitness advantages. Sugar sources are numerous, including flowers, extrafloral nectaries, sap, honeydew, and decaying fruit, but there are degrees of plant-host specialization among species. Some sources also provide secondary plant compounds that are inimical to pathogens. To locate preferred sources, biting Diptera use both visual and olfactory cues. Though many questions remain, sugar dependency offers clear opportunities to manipulate disease vectors for our benefit.

**111 Flowering plants to attract *Aedes albopictus***

Daniel L. Kline, dan.kline@ars.usda.gov, Catherine Zettel-Nalen, Eric Rohrig and Jerry Hogsette

New approaches to vector surveillance and control are being evaluated, such as the use of flowering plants. Both sexes are attracted to volatiles of nectar producing plants; these volatiles may play a significant role in host plant location. Flowering plants generate mixtures of volatile chemicals that can be exploited. The overall objective of this study is to utilize these volatiles to develop improved surveillance and control strategies for *Aedes albopictus*. Specific objectives are: determine what plant species are utilized; identify volatiles from these plants that are used for host plant location; explore potential use of these plant volatiles to develop lures for surveillance traps; and, to attract adult mosquitoes to toxic sugar bait kill stations. A survey has been conducted to identify natural and landscape plants in north central Florida that are seasonally available to our target species as a nectar source. We want to determine plant preference. Laboratory behavioral bioassays were conducted in a dual port olfactometer with selected species of flowering plants. Both intact flowers and flower extracts were used. The headspace collections of floral volatiles were analyzed by GC/MS. Current emphasis is on 2 plant species, *Buddleja davidii* (the butterfly bush) and *Solidago leavonworthii* (goldenrod). In olfactometer studies adult mosquitoes of both sexes are attracted to volatiles from both plant species.

**112 Outdoor resting and sugar feeding behaviors of African malaria vectors**

John Beier, jbeier@med.miami.edu

Studies are being conducted on the outdoor resting and sugar-feeding behaviors of *Anopheles* malaria vector populations in Mali, West Africa, where, as in most African countries, malaria is a serious public health problem. An understanding of mosquito behaviors in the outdoor environment is becoming increasingly important because there is a growing awareness that vector control tools beyond those used exclusively inside houses are needed to achieve successful malaria control and local elimination. Currently, for the major malaria vector species in Africa, *Anopheles gambiae*, *An. arabiensis*, and *An. funestus*, there is only limited knowledge on their outdoor resting behavior, and the role of sugar or nectars in their life history is mostly speculated due to a lack of field-based evidence. New field-based approaches are being used to investigate 2 important but highly neglected mosquito behaviors. By providing a strong scientific basis for development of new approaches and tools for anopheline mosquito vector control in outdoor environments, this project has strong potential for helping Mali and other countries improve malaria control and advance toward the ultimate goal of elimination.

**Operations/New Initiatives****113 Steering clear of accidents - how prevention and preparedness steps can payoff in your operation**

David McLaughlin, dmclaughlin@clarke.com and Clark Wood

One of the biggest liabilities any mosquito control program carries is the risk of an applicator accident. This paper outlines a complete plan to help prevent accidents, and how to be prepared in the event one should occur.

**114 Scientific Intelligences, is it an oxymoron or a key component of mosquito control?**

James H. Burgess, burgess@lcmcd.org and Wayne Gale

Scientific Intelligences, is it an oxymoron or a department that provides scientific data and technical information to make an effective work plan for the control of mosquitoes. This presentation will provide an overview of the Scientific Intelligences department of Lee County Mosquito Control District. A look at the variety of methods used to determine the adult mosquito population by the Scientific Intelligences including the use of laboratory support for pesticide susceptibility testing, and also conducting mosquito-borne disease testing. How the information and data it gathers daily aids in the creation of an effective work plan for the control of mosquitoes in the District will be explained.

**115 MapVision data acquisition and display, then and now**

James F. Clauson, jamesclauson@comcast.net

Beach Mosquito Control District (BMCD) is a mosquito control program located in northwest Florida. Like most modern programs, BMCD relies upon data or surveillance from the field to provide operational, managerial information decision support and to comply with state and federal regulations. BMCD accomplishes this with MapVision Enterprise software which aggregates and delivers real time data over GIS mapping and provides a robust, customized data management

system. MapVision provides reporting tools and tracking of data used to optimize control of mosquitoes such as adult and larval surveillance, lab data, LRC, material usage, Technician actions and asset tracking. MapVision provides the ability to acquire and display the necessary components needed to assist making the decisions to control both vector and nuisance mosquitoes.

**116 Florida's Indian River Lagoon: mosquito control within an estuary in distress**

Douglas Carlson, [doug.carlson@irmosquito2.org](mailto:doug.carlson@irmosquito2.org)

Along Florida's central east coast, mosquito control efforts largely focus on the saltmarsh mosquitoes produced in the Indian River Lagoon's (IRL) mangrove swamps. This is accomplished primarily through the management of 40,000 acres of impoundments and the larviciding of unimpounded marsh habitat. Over the past several years, the IRL has experienced tremendous declines in productivity including massive algal blooms, the death of approx. 60% of the seagrass acreage and high mortality of manatees, dolphins and pelicans. This presentation will provide an overview of the IRL's current problems and discuss mosquito control's role in the midst of the declining health of this "Estuary of National Significance".

**117 Strategies for managing your control material budget under variable climatic conditions**

Mark E. Smith, [mmcd\\_mes@mmcd.org](mailto:mmcd_mes@mmcd.org) and Stephen Manweiler

Changing weather patterns have caused the Metropolitan Mosquito Control District (MMCD) to reexamine control material strategies and budget allocations to maximize potential effectiveness. By maintaining flexibility in our operational plans, MMCD continues to improve our tactics and processes to better serve our citizens.

**118 District-wide resistance testing to devise site-specific control strategies**

Paula Macedo, [pmacedo@fightthebite.net](mailto:pmacedo@fightthebite.net)

Insecticide resistance has been reported for every chemical class of insecticide and therefore, mosquito populations should be periodically evaluated for their susceptibility to the pesticides used in mosquito control programs. Current susceptibility data ensures timely decisions and should be considered when adjusting control strategies. Insecticide resistance may have severe implications in the mosquito control industry. Periodic evaluation of insecticide susceptibility is part of the Sacramento-Yolo Mosquito and Vector Control District integrated vector management program. Results obtained from bottle bioassays performed in the last couple of years have been very concerning and increased tolerance to pyrethroids has been detected in various *Culex* populations. In addition, past mosquito control applications of pyrethroids in the same areas have been investigated to evaluate how much selection pressure they were contributing with, but they have been determined to be usually infrequent and not a driving force for resistance in those mosquito populations. The Sacramento-Yolo Mosquito and Vector Control District continues to evaluate various mosquito populations utilizing the bottle bioassay and will also be using microplate assays and PCR-based diagnostic tests to confirm the mechanisms of resistance.

**119 What is the Mosquito Research Foundation?**

Craig Downs, [cdowns@contracostamosquito.com](mailto:cdowns@contracostamosquito.com)

The Mosquito Research Foundation is a non-profit foundation devoted to funding needed research to enhance the understanding of the ecology of mosquitoes and the epidemiology of the diseases they transmit and develop new tools and strategies for their surveillance and control. Mosquito Research Matters!

**120 Wing Beats silver anniversary**

Stephen L. Sickerman, [sickerman@comcast.net](mailto:sickerman@comcast.net) and Jack Petersen

*Wing Beats* magazine, an official quarterly publication of AMCA and the Florida Mosquito Control Association, turns 25 this year. The Sunrise Edition, Volume 1 Number 1, premiered in Fall 1990, 20 mostly black & white pages in length. Today, *Wing Beats* is a full-color trade journal, with each 40 page issue enjoying a national and international circulation

near 4,000. An overview of the trade journal's past 2.5 decades in print will be followed with a review of manuscript submission guidelines for prospective authors.

**120a Outcomes from the USDA/ARS area-wide project for management of the Asian tiger mosquito, *Aedes albopictus***

Gary G. Clark [gary.clark@ars.usda.gov](mailto:gary.clark@ars.usda.gov), Dina M. Fonseca, Donald S. Shepard, Ary Farajollahi, Sean Healy, Yara Halasa, Kristen Bartlett-Healy, Isik Unlu, Taryn Crepeau, Sebastien Marcombe, Jiawu Xu, Daniel Kline, Randy Gaugler and Daniel Strickman

The Asian tiger mosquito, *Aedes albopictus*, became established in the continental US in 1985 and now infests 30 states. In 2007 the USDA Agricultural Research Service funded an "area-wide" project focused on the management of this species. The project was a unique federal, state, local collaboration based at the Center for Vector Biology, Rutgers University in NJ. Carefully planned surveillance and control projects were implemented and evaluated in Mercer and Monmouth Counties by local mosquito control programs. A novel aspect of this project was a partnership with economists at Brandeis University in Waltham, MA, to study the economics of local mosquito control programs and perform a cost-benefit analysis of an area-wide management program for the Asian tiger mosquito. Economists found that residents viewed mosquito control favorably and were willing to pay for enhanced mosquito control. Key results and tools are posted online at [www.rci.rutgers.edu/~AWATM](http://www.rci.rutgers.edu/~AWATM). The website contains a series of standard operating procedures (SOPs) and provides

access to over 20 publications that have already resulted from this project. There are also downloadable teaching materials and resources for costing different control strategies, as well as information on how to access a fully characterized susceptible reference strain of *Ae. albopictus* for insecticide resistance studies. The intent is to extend experiences from this project to mosquito control programs in the US and internationally.

## Vegetation Management and ATSB for Biting Fly Control II

### 121 Vegetation density in backyard and its impact on mosquito population and service requests

Rui-De Xue, xueamcd@bellsouth.net

Adult mosquitoes feed, rest, and hide in vegetation. The species of vegetation and vegetation coverage in residential backyards may affect the population of adult mosquitoes and the frequency of service requests from the residents. A survey and analysis of the frequency of service requests and the vegetation species and coverage in the residential backyards, St. Augustine, FL have been conducted based on the databases of the past 4 years and the site survey. The survey results showed that the frequency of service requests is significantly related to the vegetation coverage in the residential backyards. More service requests came from the residents who have more vegetation coverage in their backyards than the residents who have a lesser coverage. The outcome of the study benefits adult mosquito control and the improvement of customer service.

### 122 Evaluation of attractive toxic sugar baits (ATSB) mixed with pyriproxyfen sprayed on plants against container-inhabiting mosquito, *Aedes albopictus*

Rui-De Xue, xueamcd@bellsouth.net, Jodi M. Scott, Ali Fulcher, Whitney Qualls and Gunter Muller

The purpose of this experiment was to demonstrate the usefulness of combining a larvicide with an adulticide to maximize mosquito control. Attractive toxic sugar bait was mixed with 0.01% of the insect growth regulator, NyGuard® 10% pyriproxyfen EC, and applied to *Croton petra* plants in a semi-lab barrier application experiment. The treated plants were washed with 200 ml reverse osmosis water once a week for 6 wk. Ten 3-4<sup>th</sup> instar *Aedes albopictus* larvae were added to the collected wash water. The resulting emergence inhibition was monitored over a 6-wk period. The highest emergence inhibition (97%) was recorded between weeks 3 and 4. The efficacy of the ATSB/Nyguard mixture showed a slight decline (84% EI) after the sixth and final week of the plant wash. This research highlights the efficacy of adulticides in barrier applications in conjunction with larvicide triggered by rain running off the barrier treated plants into temporary bodies of water. Our results suggest positive results for the future of this synergistic approach.

### 123 New ATSB applications and active ingredients: field experiments in Israel and Mali with several mosquito species in a variety of habitats

Gunter Muller, guntermuller@hotmail.com, John Beier, V. D. Kravchenko, E. E. Revay, Rui-De Xue and Y. Schlein

Experiments were conducted with an industrial grade ATSB formulation with a natural active ingredient (EPA exempt). In Israel *Culex pipiens*, *Cx. perexiguus*, *Aedes albopictus*, *Ae. caspius* and *Anopheles sergentii* were controlled by ATSB application on vegetation. Experiments were conducted in different habitats along the Mediterranean coast and in arid habitats in the south. The impact on local mosquito populations was comparable to earlier trials using pesticides as an active ingredient. In Mali experiments are on going with a new type of bait station design. Here experiments focus on controlling mosquitoes in large outdoor cages (*Ae. aegypti*) and indoors in huts (*An. gambiae*) in combination with bed nets. Additionally impact on non-target insects is evaluated. The results will be presented in the meeting.

### 124 Evaluation of boric acid sugar bait sprayed on vegetation against salt marsh mosquito, *Aedes taenioryhynchus*

Tanjim Hossain, hossain@med.miami.edu, Ali Fulcher, Claudia Davidson, John Beier and Rui-De Xue

Boric acid toxic sugar bait (TSB, active ingredient 1% boric acid) was evaluated against *Aedes taenioryhynchus* in laboratory and field studies in St. Augustine, FL. The study sought to exploit the resting behavior of the black salt marsh mosquito on common native plants found around salt marsh habitats. Landing rate counts (LRCs) utilized in the field evaluation indicate that the areas treated with TSB presented lower LRCs than in untreated areas. Plants evaluated in the laboratory included the black mangrove (*Avicennia germinans*) and yaupon holly (*Ilex vomitoria*). These data tentatively point to limited success with boric acid TSB for exploiting resting behavior of *Ae. taenioryhynchus*.

### 125 Vegetation barrier spraying against mosquitoes in the Florida Keys

Adriane N. Tambasco, atambasco@keysmosquito.org

Beginning in May of 2012, the Florida Keys Mosquito Control District has been conducting treatment trials with a barrier sprayer, applying a residual insecticide to vegetation against nuisance and disease vectoring mosquito species in the Florida Keys. The study was performed in order to evaluate the effectiveness of a residual barrier treatment for long-range mosquito species such as *Aedes taenioryhynchus* in harborage and interception zones to determine the level of control achieved and the longevity of the treatment. Three barrier applications were conducted in northern Key Largo in 2012 to investigate whether residual barrier treatments are an appropriate technique for controlling adult mosquito populations where the option of broad scale space spraying has been reduced or removed. The trial proved this method of control to be effective at reducing the adult density of *Ae. taenioryhynchus* in adjacent residential and recreational areas so that ground ULV and aerial adulticiding were required less often. As a result of this study, the District has seen reduced pesticide application costs. Residual barrier



applications have since become an addition to the integrated mosquito management regime at the Florida Keys Mosquito Control District and new areas throughout the District are now being considered for barrier spraying.

### 126 Vegetation management and mosquito control in Volusia County, Florida

James McNelly, jmcnelly@volusia.org and Edward Northey

Volusia County Mosquito Control (VCMC) was created more than 75 years ago specifically to survey and control salt marsh mosquito species. Source reduction efforts via water management have become a cornerstone of VCMC's integrated mosquito management program, and a variety of techniques have been utilized in both tidal and freshwater wetlands, and upland discharge corridors. During the course of this work, nearly 4,000 acres of tidal wetlands were ditched or impounded, and an upland network inclusive of 190 miles of ditches and canals now exists within the VCMC district. Vegetation management to maintain ditches and canals are a significant component of VCMC's IPM program today and are performed in collaboration with Volusia County's Drainage Task Team. In partnership with St John's Water Management District, on-going restoration of tidal wetlands includes mitigation of historical source reduction spoil deposits and associated vegetation. Discussion will include the costs, approximately \$1 million annually, and benefits associated with management techniques directed at both native and non-native species such as Hydrilla (*Hydrilla verticillata*), Water Hyacinth (*Eichhornia crassipes*), Brazilian Pepper (*Schinus terebinthifolius*) and Australian Pine (*Casuarina spp*).

### 127 Managing overgrown vegetation to tame the Asian tiger mosquito in an urban jungle

Isik Unlu, ioguzoglu@hotmail.com , Nick Indelicato, Brittany Cerino and Ary Farajollahi

*Aedes albopictus* (Skuse) is recognized as a major pest because of its diurnal and aggressive anthropophilic host preference behavior. This mosquito is also a public health concern because of its ability to vector many arboviruses, including dengue and chikungunya. In regions where *Ae. albopictus* has become established, there is a growing demand for an efficient and cost-effective panel of control strategies for suppression of this species. Our study was conducted in 6 parcels (individual home and accompanying backyard) in an area with high numbers of *Ae. albopictus* adults in the City of Trenton, Mercer County, NJ. All parcels were categorized as abandoned and poorly maintained by city officials. We selected 3 parcels as controls to only monitor *Ae. albopictus* abundance by BGS traps and 3 parcels as treatments to cut over-grown vegetation in order to eliminate mosquito resting areas and thus adult populations. We were able to reduce the average number of adult *Ae. albopictus* for 8 days (3 locations), with an average percent reduction varying between 16% -96%. We discuss the implications of vegetation management within an integrated mosquito management program aimed at the Asian tiger mosquito.

## Behavior and Biology

### 128 Are tigers in a concrete jungle really diurnal? Diel activity of *Aedes albopictus* determined through human sweep net and rotating trap collections

Ary Farajollahi, afarajollahi@mercercounty.org, James McNelly, Isik Unlu, Nick Indelicato, Garrett Dow, Randy Gaugler and Dina M. Fonseca

*Aedes albopictus*, the Asian tiger mosquito (ATM), has been traditionally considered a diurnal species which primarily feeds in the daytime, rarely at night. The diurnal biting activity of ATMs has been observed to be generally bimodal, with a peak in activity during the early morning and another in the evening. However, our recent investigations with human sweep net collections and cyclical rotating traps provide evidence that in the northernmost extent of ATM range in northeastern USA, these species behave as ambush predators and may feed readily during the day or night.

### 129 Anatomy of mosquito larvae from a submariner's perspective

Herbert Nyberg, sales@newmountain.com

The scientific investigation of the mechanism of mortality from acoustic larvicide<sup>®</sup> revealed many interesting characteristics of the internal anatomy of mosquito larvae that had not previously been studied. In particular the dorsal tracheal trunk was identified as a very high pressure organ, and its construction has interesting impacts on the overall physiology and morphology of the larvae. It has also been observed that many internal and external biological structures of mosquito larvae have corresponding structures in submarine designs. This presentation discusses in detail the dorsal tracheal trunk structure and its use as well as the interesting comparison between larvae and submarine design.

### 130 Investigating dynamics of the bacterium *Asaia*, a potential paratransgenic disease control method, within *Aedes albopictus*

Elizabeth S. Andrews, elizabeth.s.andrews11.ctr@mail.mil and Stephen L. Dobson

*Asaia*, an acetic acid bacterium, has gained interest as a potential novel method of paratransgenic mosquito-borne disease control because of its direct association with mosquitoes of medical importance. To determine if the bacterium is an associate of *Aedes albopictus*, *Asaia* infection dynamics were investigated. The microbial community within *Ae. albopictus* was compared between field-collected and laboratory-reared females and their eggs using DGGE. *Asaia* was detected within both types of females, but not within egg pools. Culturing *Asaia* from field-collected mosquitoes yielded 2 genetically different isolates, both of which are >99% identical with those isolated in previous studies. Field experiments involved

collection of wild populations of *Ae. albopictus* and flowering plants as possible sources of *Asaia*. These experiments determined that there was seasonal and yearly variation in our ability to detect *Asaia* in the environment. Laboratory experiments observed that the bacterium was continuously associated with *Ae. albopictus* for the duration of its lifespan, although rates of infection within the population were not 100%. *Asaia* was present in midguts and testes, but rarely ovaries, possibly indicating a lack of vertical transmission routes. The results indicate that, although *Asaia* may associate with *Ae. albopictus*, it may not be a facultative symbiont, as observed in other mosquito species, and may be opportunistically acquired from the mosquito's environment.

### 131 Oviposition of container-breeding mosquitoes associated with water treated with ORIGIN 3-6-9

Richard Duhrkopf, rick\_duhrkopf@baylor.edu and Alyssa Pantle

Ovitraps were used to assess whether females will lay eggs in water containing the chemical ORIGIN 3-6-9.

### 132 Adulticide resistance profiles in *Culex quinquefasciatus* populations from Mississippi 2013

Mariah Scott, gni6@cdc.gov, Wendy Varnado and Janet McAllister

To manage vector-borne diseases, which often have no treatment, vaccine or prophylactics available, vector control is the only viable option. To achieve good control of vector populations the use of effective insecticides is essential. If insecticides are used inappropriately or not administered correctly, then insecticide resistance can develop. The state of Mississippi is comprised of 82 counties, with each county or cities within the county independently responsible for vector control. This leads to a variety of approaches being employed, including different control practices and insecticides applied. The Mississippi State Health Department and the Centers for Disease Control and Prevention (CDC) collaborated on a project to determine the resistance profiles in 9 *Culex quinquefasciatus* populations between Jackson and Hattiesburg. CDC performed bottle, biochemical, and molecular assays to detect the presence of resistance and characterize the mechanisms involved. Overall, there was minor malathion resistance detected at 2 sites, however in 2 sites, over 50% of the population exhibited permethrin resistance. Glutathione *S*-transferases (GST) and esterases played a role in the malathion resistance with minor oxidase involvement. GST played a major role in permethrin resistance along with oxidases and esterases. Although minimal to no permethrin resistance was seen in 5 of the populations, the knockdown resistance (*kdr*) mutation was present.

### 133 Bite protection analysis of permethrin-treated US military combat uniforms

Ulrich R. Bernier, uli.bernier@ars.usda.gov, Melynda K. Perry and Amy L. Johnson

Historically, casualties from diseases have greatly outnumbered those from combat during military operations. Since 1951, US military combat uniforms have been chemically treated to protect personnel from arthropod attack. In the 1970s and 1980s, permethrin was one of several insecticides evaluated as a repellent treatment for uniforms. In 1991, permethrin became the standard treatment of US military combat uniforms. In 2007 the US Marine Corps transitioned from treatment with permethrin in the field to factory treatment of their 50/50 nylon/cotton Marine Corps Combat Utility Uniforms (MCCUUs). The US Army transitioned to factory treatment of uniforms in 2009. Over the past few years, an increasing proportion of combat uniforms is constructed from fabric comprised of nylon, rayon and fire resistant materials such as para-aramid or meta-aramid. These uniforms cannot be treated with permethrin in the field and must therefore be treated at the factory level. Incorporation of permethrin into the fabric significantly reduces the probability that a mosquito can bite through the uniform. Results from bite protection studies will be covered in this presentation. The emphasis will be on the performance of the newest fire-resistant uniforms, these include the US Marine Corps Enhanced Fire Resistant Combat Ensemble (EFRCE) and the US Army Fire Resistant Army Combat Uniform (FRACU) and FRACU type III.

### 134 Correlation of lipophilicity with mosquito larvicidal and repellent activities of novel trifluoromethylphenylamides

Maia Tsikolia, maia.tsikolia@ARS.USDA.GOV, Ulrich R. Bernier, Natasha M. Agramonte, Monique R. Coy, James J. Becnel, Gary G. Clark and Kenneth J. Linthicum

For a chemical to have a biological effect on an organism, the molecules must be capable of being transported across the membranes of cells. Lipophilic insecticides that can pass through lipid bilayers and penetrate the insect cuticle can lead to rapid intoxication or mortality by acting on the nervous system. LogP values are a measurement of a partition between 2 phases (octanol and water) for a unionized (neutral) compound. This study explores the relationship between lipophilicity (in terms of LogP values), and larvicidal and repellent activities against *Aedes aegypti* for a series of similarly structured compounds. For 20 trifluoromethylphenyl amides, the LogP values were calculated and compared to larvicidal and repellency bioassay results. Calculation of LogP was accomplished by using the virtual computational software programs, Molinspiration and ALOGPS. We observed that compounds with higher LogP (4.0-5.1) values, exhibited more potent larvicidal activity although compounds that exceeded a certain level of lipophilicity (LogP~5.3) had decreased larvicidal activity. We observed an increase in repellent activity with increasing lipophilicity (LogP from ~4 to ~5). However, lipophilicity of the most active repellents within this set had LogP values that ranged from 2.8-4.4. In conclusion, this study indicates that the lipophilicity coefficient may be an important property to consider in the design of insecticide and repellent candidates.

**135 The Asian tiger mosquito, *Aedes albopictus* in southeastern Massachusetts**

Priscilla Matton, brismosqpc@comcast.net

The Asian tiger mosquito, *Aedes albopictus* has been collected from 1 site in southeastern Massachusetts routinely since 2008. BG-Sentinel™ mosquito traps were used to determine the regional distribution in the area during the 2013 mosquito season.

**136 Genetic diversity in the dengue mosquito *Aedes aegypti* (Diptera: Culicidae) from Punjab, Pakistan using RAPD/Microsatellite markers**

Shabab Nasir, flourenceshabab@yahoo.com

The mosquito *Aedes aegypti* is the main vector of dengue. Although the epidemiology of dengue fever has been widely described, the genetics of the dengue vector mosquito remained poorly understood in Pakistan. Hence, in order to estimate the genetic variability in *Ae. aegypti* populations in different areas of Punjab, random amplified polymorphic DNA and microsatellite markers were used. *Aedes aegypti* mosquitoes were collected by using standard techniques (dippers) from Faisalabad, Nankana Sahib and Lahore districts. The collected populations from different cities were reared separately in the laboratory and then DNA was extracted from single individuals respectively. DNA concentration was measured by using electrophoresis standards after the extraction. Ten decamer random primers (GL Decamer A-01 to GL Decamer A-10) were selected for polymerase chain reaction (PCR). The PCR products were analyzed using 1.5% agarose gel electrophoresis. The amplification profile data showed monomorphism and polymorphism between different districts as well as between samples of even the same district. The primer A-04 showed a clear polymorphic banding pattern in different *Ae. aegypti* samples. Based on the inter- and intra-populations polymorphic data from the current findings of all 3 districts, it is concluded that *Ae. aegypti* populations were slightly genetically different in selected areas.

**137 Field monitoring of potential effects of *Bacillus thuringiensis* subspecies *israelensis* (Bti) on the honeybee *Apis mellifera* in western France**

Frank Aletru, smo.inter@wanadoo.fr, Jérôme de Maupéou and Sébastien Chouin

Because of European Community regulations, mosquito control programs must evaluate potential effects of biocide treatments on fauna and flora registered in a particular environmental network named Natura 2000. Since 2010, EID Atlantique board of directors, composed by elected people from 5 counties, decided to determine if larval treatments with *Bacillus thuringiensis* subspecies *israelensis* (Bti) used to regulate mosquito populations are safe. From 2011 to 2013, a field study was conducted to observe potential effects of treatments on the honeybee *Apis mellifera*. Associated with the French National Center of Scientific Research (CNRS) the Vendéen Center for Research on Honeybee (CVRSA), implemented specific field monitoring at 5 sites located close to mosquito ovipositing sites regularly treated with VectoBac WG. Each study site was composed of 4 beehives installed on weighing machine connected and remotely interrogatable. This beehive network was compared with a non-treated one. During this study, the foraging activity and behavior of bees was observed. The growth of the colonies was noted and mortality measures were taken. At the end, no adverse effect of *Bacillus thuringiensis* subspecies *israelensis* (VectoBac WG) was observed.

**138 Factors influencing the reproductive potential of *Culex pipiens* during prolonged periods of oviposition site deprivation**

Brian Johnson, brijo85@eden.rutgers.edu and Dina M. Fonseca

High rates of West Nile virus (WNV) transmission to humans are associated with exceptionally hot and dry summers. This is paradoxical since *Culex* vectors of WNV depend on water containers for larval development. We hypothesized that forced-egg retention in *Culex pipiens* may induce additional blood-feeding resulting in higher arboviral transmission. We examined the effects of forced-egg retention with additional blood sources on the reproductive success of *Cx. pipiens* for a period of up to 7 wk. To address confounding effects we also controlled for female and sperm age. Increases in the length of forced-egg retention resulted in significant decreases in fecundity (number of eggs oviposited) and fertility (number of larvae hatched/egg raft) but the decline was moderate for 5 wk. Importantly, no females took blood more than once per gonotrophic cycle and surprisingly, increases in female age at the onset of the first gonotrophic cycle were associated with increases in fertility. We conclude that consistent with an "all-or-none" ovipositing strategy, *Cx. pipiens* females can withstand long periods of forced-egg retention without major reproductive costs. A corollary of these findings is that large mosquito populations may congregate near reduced water sources and potentiate WNV transmission by increasing the contact between vector and host.

**Highlights of Mosquito Control**

**139 Highlights of mosquito biology: 2013**

Seth C. Britch, seth.britch@ars.usda.gov

This presentation will summarize the latest advancements in mosquito research, based on papers published in 2013. The objective is to provide insight synthesized across a full spectrum of disciplines that we may not typically encounter in literature surveys within our individual concentrations. This highlights section will review the year's research in mosquitoes as organisms, including, for example, their behavior, morphology, physiology, genetics, phylogeny, and individual and

population ecology, and also research in mosquitoes from the point of view of their role in the epidemiology of associated pathogens such as viruses, filarial worms, and parasites, including effects of changing climate, and the array of traditional and novel countermeasures. Although a broad set of topics will be reviewed, the aspects most relevant to medical and veterinary entomology and mosquito control, for example new discoveries in behavior or physiology leveraged to improve mosquito surveillance, will be covered with greater detail. The content of the review will indicate the recent key engagements of the research community, but may also help us identify neglected subjects or form new questions.

#### 140 Highlights of biting midge biology: 2013

Lee W. Cohnstaedt, Lee.Cohnstaedt@ars.usda.gov

Recent outbreaks of bluetongue-8 (BTV8) and Schmallenberg viruses in Europe and epizootic hemorrhagic disease (EHD) in the United States have increased scientific interest in biting midge (*Culicoides*) disease vectors. Reviewed in this presentation are the latest advancements in *Culicoides* taxonomy, ecology, behavior, midge/disease surveillance, pathogen transmission, and epidemiology. The manuscripts were selected based on citation number and likely effect on the field. The titles of all 200+ articles published world-wide on biting midges in 2013 can be found on "The Ceratopogonidae Information Exchange", which is maintained by Dr. Steven Murphree at Belmont University. Topics will be broadly reviewed and their relationship to disease vector control and epidemiology emphasized. Ground-breaking manuscripts will be discussed in depth and interesting or bizarre findings will be noted. The goal of this review is to provide insight into the latest Ceratopogonidae research topics and direction.

#### 141 Highlights of control technology: 2013

Mark Breidenbaugh, mbreiden@kent.edu

Following the tradition of "Highlights Symposia" in other professional associations, the published literature describing mosquito control technologies in 2013 was reviewed. From these works, a selection of publications was chosen to be "highlighted" by describing the authors' various methods and findings as they relate to mosquito control technologies both from an academic perspective as well as from that of the grass roots technician.

### Larval Control

#### 142 Comparison of single brood larvicides: *Bti* and spinosad

Ann Moser, amoser@gmcdl.org

A large-scale field study performed in Grant County, WA compared the length of control from *Bti* and spinosad, 2 single brood larvicides. Calibrated aircraft applied the larvicides and control was evaluated for 30 days. Comparison of data showed *Bti* gave single brood control while spinosad had a residual effect against mosquito larvae.

#### 143 Optimizing granule formulation applications of Natular® larvicide

Jake Hartle, jhartle@clarke.com and David McLaughlin

In the 5 years since introduction, much has been learned about operational application of Natular® G and G30 granule formulations. This paper will discuss a collective learning of the key factors to consider for successful application from habitat and rates to application methods and timing.

#### 144 Spring larvicide application of VectoBac 12AS and VectoLex WDG in an Atlantic white cedar (*Chamaecyparis thyoides*) swamp in southeastern Massachusetts

Ellen Bidlack, ebidlack@plymouthmosquito.org and Priscilla Matton

Control of mosquito larvae in Atlantic white cedar swamps can be problematic. The challenge is generally 2-fold, the thick canopy is difficult to penetrate and the swamp can produce multiple broods of larvae that cannot be killed with 1 application of *Bti* (*Bacillus thuringiensis israelensis*). We tested the ability of a low volume application of VectoBac 12AS and VectoLex WDG to penetrate the canopy of an Atlantic white cedar swamp. We applied 1 pint/acre of VectoBac 12AS and 0.175 lb/ acre of VectoLex WDG to a 140-acre swamp using a Bell Jet Ranger helicopter. Efficacy was evaluated using fixed dip stations and a cup bioassay developed by the Center for Vector Biology at Rutgers the State University of New Jersey. The dip stations were monitored for 2 wk post-application, and mosquito production was compared to a control site. The cups were brought back to the laboratory where *Culex pipiens* larvae were added to the cups and monitored for mortality and re-circulation of *Bacillus sphaericus*.

#### 145 Larval source management as part of the malaria vector control in West Africa

Mavy H. Rodriguez, mavygaby05@yahoo.com, Sandra M. Reyes, Adam H. Taher, Yudit Hernández, Abel G. Avila, Lisset Morell, Pierre Kayende, Conrado Vazquez, Fernando Vecino, Kalada Irinuarebe and Felix Quintanar

The control of malaria in Africa remains a challenge for public health systems. The 2012 World Malaria Report of WHO reflects that a 25 % reduction in malaria cases has been achieved; however, the disease still is the primary cause of death in 19 countries of the world. Integrated vector control is one of the ways to accelerate the roll-back of malaria. The inclusion of strategies such as larval source management in Africa is a controversial theme for health authorities. The purpose of this work is to demonstrate the effect of the biological larvicides *Bacillus sphaericus* 2362 and *Bacillus thuringiensis israelensis*

to control *Anopheles gambiae* and *Anopheles funestus* complex in countries of West Africa. Aqueous formulations of *Bti* and *Bs* with a dosage of 2-5 ml/m<sup>2</sup> and 5-10 ml/m<sup>2</sup>, respectively, were applied in heterogeneous oviposition sites in urban and rural areas in localities of Ghana, Nigeria and Burkina Faso. Entomological surveillance was established according to the rainy and dry seasons to monitor the monthly vector behavior (larvae and adults). In all cases, a decrease higher than 90 % in the relative larval density as well as a 70 % reduction of the adult mosquito density was obtained. The effectiveness of this method and its feasibility to be applied in urban and rural areas of Africa were clearly evidenced.

### 146 Bioactivity of medicinal plants extracts against dengue mosquito *Aedes aegypti* (Diptera: Culicidae)

Shabab Nasir, flourenceshabab@yahoo.com

This study was performed to evaluate the larvicidal and pupicidal potential of the essential oils extracted from the branches and leaves of eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), peppermint (*Mentha spicata*), niazbow (*Ocimum basilicum*) and from the rhizome of ginger (*Kaempferia galangal*) against the larval and pupal stages of *Aedes aegypti*. The essential oils were extracted by using soxhlet apparatus with petroleum ether as a solvent. The oils were evaluated against 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar larvae and pupae of *Aedes aegypti* using WHO protocol. The dead were counted after 8, 16, 24 and 48 h with different concentrations (100, 200, 300 and 400 ppm), and LC<sub>50</sub> and LT<sub>50</sub> values were also calculated. A control treatment was also run by using petroleum ether. Petroleum ether also showed a great effect on the mortality of different life stages of *Aedes* mosquitoes. Higher mortality was observed in early life stages than late life stages. Different concentrations of eucalyptus oil showed greater mortality in different life stages than other oils. All the oils were statistically at par with each other. The ginger oil showed the least mortality in case of different life stages. All the studied factors and their interactions were found highly statistically significant.

### 147 Determination of the LC<sub>50</sub> for the use of ORIGIN 3-6-9 as a larvicide

Richard Duhrkopf, rick\_duhrkopf@baylor.edu

ORIGIN 3-6-9 has been demonstrated to be an effective larvicidal agent. This study tested larvicidal activity in water containing various concentrations of ORIGIN 3-6-9 to determine the LC<sub>50</sub> for the chemical.

### 148 Larviciding as an alternative for the integrated vector management for malaria control in urban and sub-urban areas in Angola

Roberto S. Prieto, robertosanchez1658@gmail.com, Filomeno Fortes, Celia Roca and Jesús Joya

Entomological and epidemiological surveys were conducted in 7 provinces of Angola (Luanda, Benguela, Cabinda, Cunene, Huambo, Kwanza Norte and Namibe) represented by 18 localities, with the objective of evaluating the strategy of integrated vector management by means of application of biolarvicides to control the transmitting vectors of malaria and of other diseases. The localization and mapping the oviposition sites was established and larval density was determined before and after treatment using the dipper method. The knock-down technique was employed to determine mosquito average density per house, as well as the taxonomic classification by classical methods of morphologic identification of *Anopheles* spp. Physiological age was determined with the application of Detinova's technique and the sporozoitic index. A parasitological inquiry was conducted in 4,738 children of ages between 6 months and 14 years. The prevalence rate oscillated between 0.001 and 0.09. A noticeable reduction in larvae and adults density of the vector was achieved. The biolarvicides effectiveness (*Bacillus thuringiensis israelensis* and *Bacillus sphaericus* strain 2362) was of 99.6 and 100% respectively. *Anopheles gambiae* and *An. funestus* complex were identified in the monitored provinces. The morbidity decrease in 2012, in comparison with 2009, proved that biological larval control must be a reinforcement intervention in all integrated malaria control programs.

### 149 Evaluation of methionine, an essential amino acid, as a mosquito larvicide

Emma Weeks, eniweeks@ufl.edu, Sandra Allan, Julie Baniszewski, Alissa Berro, Jim Cuda and Bruce Stevens

Mosquito larvicides are an effective means of source reduction, controlling the population size so that the number of adult females that are present to bite and potentially spread pathogenic organisms is decreased. Currently utilized mosquito larvicides include insect growth regulators, organophosphates, oils, and microbial agents. As resistance has developed to the organophosphate (e.g., temephos) and microbial (e.g., *Bacillus thuringiensis israelensis*) larvicides that are commonly used, alternatives are urgently needed. Our study aims to evaluate the toxicity of methionine, an essential amino acid, as a mosquito larvicide alone and in combination with existing larval control techniques. To date efficacy of methionine as a larvicide against all larval instars of *Aedes albopictus* and *Anopheles quadrimaculatus* has been demonstrated. Testing with *Culex tarsalis* is currently in progress. Providing that the effect on non-target organisms is minimal, methionine could provide a valuable addition to an integrated mosquito management program.

### 150 Aquatain AMF: summary of efficacy trials

Graham Strachan, graham@aquatain.com

Aquatain AMF is a silicone based liquid for mosquito control. It spreads strongly across the surface of standing water, forming a very thin film. As the silicone polymer has a low surface tension, larvae and pupae cannot attach to the surface to breathe, causing them to drown. Many trials have been conducted with the product around the world, and this paper presents an overview of the results. As well as peer-reviewed studies by Wageningen University, Sydney University and the Kenya

Medical Research Institute, trials have been undertaken by several ministries of health, universities and government bodies. The trials have been conducted on the 3 major species of mosquitoes, *Anopheles*, *Aedes* and *Culex*; and the main conclusions are that: 1) the pupae of all species die within about 2 h; 2) the larvae also die, but the young larvae of some species may survive for a few days; 3) there is virtually no egg laying when the film is in place; and 4) the film is effective for 4 wk on the surface. These attributes make the product superior to traditional surface films. The impact on *Culex* pupae and larvae is particularly rapid, which is of interest in view of the emerging threat of West Nile virus. Silicones have been extensively studied for over 50 years, and they do not present a risk to the environment or to human health.

## GIS and Risk Assessment

### 151 Implementation of Sentinel™ GIS for NPDES compliance - Gem County Mosquito Abatement District

Jason R. Kinley, director@gcmad.org

The Gem County Mosquito Abatement District implemented Sentinel™ GIS for NPDES permit compliance. The use of Sentinel™ GIS allowed the district to accurately report the amount of mosquito control products applied to waters of the United States. The data were used to complete annual usage reports and to query information for different needs. The process used to implement Sentinel™ GIS for NPDES compliance will be discussed.

### 152 Distributed GIS: leveraging GIS technology throughout the entire organization

Ryan D. Pierson, ryan@elecdata.com

Geographic information system (GIS) technology is utilized by many organizations, but typically a limited few within an organization regularly access and use this technology. To make GIS technology more useful and more valuable to the organization, it is important that GIS data and tools are distributed throughout the entire organization. This always is true for managerial and office staff, but now GIS technology can easily be extended to the field workforce too. This presentation will provide an overview of GIS implementation that distributes GIS technology and tools to an entire workforce, whether working in the office at a PC or working from an ATV in the field. Examples of real-time GIS interaction and disconnected GIS options will be presented. Specifically, this presentation will provide examples of distributing GIS technology and tools to aid in mosquito control operations. Distributing GIS tools throughout the organization can increase operational efficiency while reducing operational costs.

### 153 Modeling risk of exposure to malaria vectors

Temitope O. Alimi, t.alimi@umiami.edu

The public health problem posed by malaria has made it a top priority for control and elimination efforts. Consequently, interventions are ongoing including in northern South America (NSA). We set out to investigate the distribution of transmission risk in NSA by producing risk maps that identify risk areas so that they may be targeted. Spatial data layers of environmental and anthropogenic parameters that influence mosquito distribution were combined in a multi-criteria evaluation model to map risk of vectorial exposure. Distance layers were created for discrete factors and all factors were standardized on a byte 0-255 probability scale using a fuzzy function. Weights were generated based on expert opinions and assigned using analytical hierarchy process. Risk maps were validated using data on *Anopheles darlingi*, *An. nuneztovari*, and *An. albimanus*. Risk scores for mosquito occurrence points were significantly different from those generated randomly, and *An. darlingi* consistently had higher risk scores compared to *An. nuneztovari* and *An. albimanus*, a probable indication of its competence in malaria transmission. Prioritization of strategic control interventions in relevant foci of malaria transmission could be enhanced through risk mapping as interventions would be administered based on stratification of risk.

### 154 Decreasing response time to adult mosquito population and arbovirus surveillance results

Gregory White, gwhite@cvmvcd.org and Marko Petrovic

Surveillance for adult mosquito abundance and for the presence of vector-borne diseases is used to direct mosquito control operations. How effective the operational response is to surveillance results, such as high trap counts of vector species, or arbovirus positive mosquito pools is greatly determined by the response time to surveillance data. The Coachella Valley Mosquito and Vector Control District created a software application to rapidly assess the status of all known larval sources in a user determined radius. This allowed the District to quickly respond to surveillance data during the 2013 mosquito season. The utility of this program was especially helpful in urban areas where West Nile virus was detected and where there was a high density of larval sources. This became especially important during 2013 as the District faced a 200-year rain event, which created many new sources and disrupted treatments at other known sites. The inspection and treatment history of all sources was made quickly available to supervisors and technicians and divided into quadrants for easy division of labor if necessary. Later modifications to the program were then made using the treatment and larval inspection data to assist in adult mosquito trap placement. Overall the new program has increased the speed of our mosquito control actions to our surveillance data, increasing the District's capability to control mosquito populations and interrupt arbovirus transmission.

### 155 Enterprise mobile web app in the cloud - from buzzwords to reality

Nancy Read, nancread@mmcd.org and Brian Fischer

The Metropolitan Mosquito Control District (MMCD) manages work done in 70,000 wetlands in the Minneapolis/St. Paul metro area. With the help of Houston Engineering, Inc., we have been moving from dispersed data and proprietary map

software into a centralized enterprise system built from open source software and accessible from any device with a web browser. This presentation will give an overview of our move, and development of field data entry for smart phones that also works on PCs. This enterprise system is being used to manage and integrate surveillance, inspections, treatments, citizen requests, and helicopter tracking activities, and to pull in weather data from additional sources. It also has enabled MMCD to make data available directly to citizens through web map access.

### 156 Performing mosquito control using MobileVC GIS tool

Dan Damian, DDamian@mail.maricopa.gov, Kirk Smith and John Townsend

This presentation will focus on collaborative efforts to capture, store, and analyze field collected surveillance data across Maricopa County, AZ. Together as part of 5 functional groups we have successfully centralized environmental health spatial and tabular information into an enterprise SDE database.

## Invasion: When Exotics Visit and Stay

### 157 Features of the current wave of biological invasions

Anthony J. Cornel, cornel@uckac.edu

Before mass migrations of humans, animal and plant dispersal was restricted by geographic barriers such as oceans, mountain ranges, and deserts. The barriers have been lowered by human activity, particularly at the end of the Quaternary glacial period. These days, almost every location on the planet has been invaded by species that originated elsewhere. There are multiple examples of non-deliberate invasions of mosquitoes such as *Aedes/Stegomyia albopicta*, *Aedes/Stegomyia aegypti* and *Culex quinquefasciatus* and its sibling species. There are some traits associated with invasive species that make them successful invaders and these traits will be discussed. In this symposium we will focus on invasion of specific species that have had public health and economic implications. Invasion by alien species, along with climate change and habitat clearance, will likely eventually assemble natural communities dominated by few species and lower overall diversity of mosquito species.

### 158 *Aedes albopictus* in Los Angeles County, California, USA. Have we caught a tiger by the tail?

Kenn K. Fujioka, kfujioka@sgvmosquito.org, Joseph W. Wekesa, Kelly M. Middleton, Susanne Klueh, Mark Daniel and Teresa E. Sorvillo

In September 2011, a population of *Aedes albopictus* was identified in the cities of El Monte and South El Monte, Los Angeles County, CA. Previous infestations of *Ae. albopictus* in the Los Angeles Basin in 2001 and 2004 were successfully eradicated; this led vector control agencies in the area to surmise that environmental conditions were not conducive for *Ae. albopictus* to rapidly proliferate and survive. The Greater Los Angeles County Vector Control District and the San Gabriel Valley Mosquito and Vector Control District received assistance from many local, county, state, and federal agencies as we struggled to identify the scope of the infestation and develop a strategy to eradicate *Ae. albopictus* from the area. We continue to evaluate our experiences and those of others as we contemplate the future and wonder whether we will successfully remove *Ae. albopictus* or be forced to manage it in our jurisdictions.

### 159 Albos down under: from vector to BBQ killer

Scott Ritchie, scott.ritchie@jcu.edu.au

The Asian tiger mosquito, aka "albo", *Aedes albopictus* was first detected in several Torres Strait islands in north Queensland, Australia, in 2005. Since this detection, the Australian Commonwealth, through Queensland Health, have developed an *Aedes albopictus* Control Program. Initially, this program aimed to eliminate albos from the Torres Strait. Then, following a reality check, the program established a red line "cordon sanitaire" to minimize populations on the primary transport hubs to mainland Australia. The goal of the program is to minimize the risk of invasion of the mainland by strong vector control in the Torres Strait. The current program relies upon source reduction and larviciding, and use of harborage and barrier spraying with synthetic pyrethroids to control adult populations. The latter approach has been very effective at controlling adult populations, and we have reduced albo populations on treated islands by > 90% over initial populations. We have also developed unique sampling methods that will be discussed.

### 160 Aussie Mozzie (*Aedes camptorhynchus*, the southern salt marsh mosquito) invades New Zealand. And the Kiwi's fight back

David Sullivan, zanusco1@msn.com

In December 1998, vicious day-biting mosquitoes threatened to exsanguinate the residents of Hawkes Bay, on the east coast of New Zealand's North Island. Further investigation found that the mosquito was *Aedes camptorhynchus* (Campto) the southern salt marsh mosquito of Australia. Campto was known to transmit Ross River virus in Australia and since the climate in Hawkes Bay was similar to the natural habitat of Campto in Australia, the New Zealand Health Authorities initiated a plan to limit the advance of this invasive species. The potential health risks of getting Ross River virus in New Zealand were estimated to cost from NZ\$ 12 million to NZ\$ 39 million every 3 to 5 years with cyclical epidemics, if Campto were established in NZ. A Technical Advisory Group (TAG) was formed in January 1999, which included experts from Austria and New Zealand. The TAG team concluded that an eradication attempt was possible if they could use a product that

had a long-term residual and was safe for the environment, such as granule and pellet formulations of S-methoprene (Altosid®), which had been used in Australia for a number of years. Zanus Corporation was able to register the S-methoprene granules and pellets in NZ with a ProLink brand. Treatments started in 1999 and continued until 2006 when Campito was declared eradicated.

#### 161 Exotic mosquitoes conquer Europe

Nobert Becker, nobertbecker@web.de, Arthur Jöst, Carola Kuhn, Andreas Krüger, Andreas Rose, Francis Schaffner, Peter Lüthy and Egbert Tannich

Increased mobility of humans, as well as international trade, facilitates the dispersal and in some cases, the establishment of exotic mosquito species outside their original area. Three species merit special mention regarding their dispersal potential and also for their significance as vectors of human diseases, *Aedes aegypti*, *Ae. albopictus*, and *Ochlerotatus japonicus*. In Europe other exotic species have been introduced and established, *Oc. koreicus*, *Oc. atropalpus* and *Oc. triseriatus*. Once established, these species can be spread by vehicles, trains, and even boats to neighboring regions and countries. *Aedes aegypti* was found, prior to 1945, in all Mediterranean countries. Recently, *Ae. aegypti* has been introduced into the Netherlands and is established in Madeira and around the Black Sea in southern Russia, Abkhazia, and Georgia. *Aedes albopictus* is the most problematic species. In the early 1990s, it was passively introduced into Italy as a result of the international trade in used tires. Since 1999, *Ae. albopictus* has been found in most southern and central European countries. *Ochlerotatus j. japonicus* has been recorded in central European countries, including several states of Germany, since 2000. WHO and EMCA have developed "Guidelines for the control of invasive mosquitoes and associated vector-borne diseases on the European continent." In Germany the rapid spread of *Ae. albopictus* and *Oc. japonicus* increased awareness and has led to an intensive surveillance program for exotic mosquito species.

#### 162 What are you doing here? *Aedes aegypti* in the Central Valley of California

Jodi J. Holeman, jholeman@mosquitobuzz.net

*Aedes aegypti*, the principal vector of yellow fever, dengue and chikungunya viruses, was discovered in the Central Valley of California (Fresno and Madera counties) during June 2013. This represents the first time this species has been detected in the interior of California and the first record of adults collected in the state. The mosquito was subsequently found in San Mateo County in August. In response to this invasion, the Consolidated Mosquito Abatement District (District) in Fresno County developed a spatially driven *Ae. aegypti* control program based on surveillance data. The program was developed within the District's current surveillance and control geographic information system (GIS). Changes to this system were necessary to accommodate the unique challenges that controlling *Ae. aegypti* present. Mosquito control activities undertaken in response to the detection included trapping and control methodologies and strategies (both standard and novel) and public outreach and awareness campaigns, as well as information mining and collaborations with UC researchers. The detection of *Ae. aegypti* and other exotic mosquitoes in areas considered outside their normal ranges and habitats reiterates the need for development of preparedness and response plans for the introduction of exotic vectors and emerging diseases.

### Larval Control/Novel Methods, Products

#### 163 Auto-dissemination augmented by males (ADAM) to control *Aedes albopictus*

Corey Brelsfoard, cbrelsfoard@mosquitomate.com, James W. Mains and Stephen L. Dobson

Mosquito control remains the only means available to combat many medically important and nuisance mosquito species. Chemical insecticides are most commonly used to control mosquitoes, with formulations that include larvicides and adulticides. While larvicides have been proven effective at reducing mosquito-borne disease transmission, its implementation at the programmatic level can be difficult. The aquatic habitats of many mosquito species, including the Asian tiger mosquito (*Aedes albopictus*), can be small and difficult to locate and treat, sometimes referred to as 'cryptic oviposition sites'. Here, we will present initial results of an auto dissemination approach as a method of pesticide 'self-delivery' by the mosquito to cryptic oviposition sites. The results suggest the feasibility of an auto dissemination approach, where adult males are treated with pyriproxyfen (PPF) (Auto Dissemination by Males - ADAM). The data suggests that: 1) adult male mosquitoes can be treated with PPF levels that do not effect survivorship or affect their ability to mate or interact with females; 2) treated males can deliver lethal concentrations of PPF directly to larval development sites; and 3) treated males can transfer PPF to females, which can then subsequently transfer lethal levels of PPF to larval development sites. This report will also briefly summarize plans for additional work to test the ADAM approach as a mosquito control tool.

#### 164 Mycological control of mosquito vectors: a natural technology

Gavendra Singh, singhgavendra@gmail.com and Soam Prakash

Mycological controls of mosquitoes are a key element in most malaria management programs. The fungal bioproducts have generated high rates of mortality. In the present investigation, fungal metabolites of *Fusarium solani*, *Memmoniella levispora* and *Culicinomyces clavisporus* have been purified with chromatography techniques to enhance efficacy. These purified products have been tested against developmental stages of larvae and adults of *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Between 90 - 95% mortality was observed against larvae and adults of *An. stephensi* and *Cx. quinquefasciatus* after 24-h exposure. Moreover, we also observed 100% mortality of *Ae. aegypti* larvae after 24-h exposure.



The bioactive compounds have been detected in a thin layer chromatography study. This study clearly demonstrated that the mycomolecules can be a “green” natural alternative to chemically -based restricted larvicides and adulticides restricted. These results show that mycological control can be used successfully in integrated vector control programs.

### 165 Operationalizing the use of biological larvicides as a new intervention for malaria vector control in Zambia

Aramis Martinez, amarias2010@yahoo.com, Tamara Gutierrez, Alberto Escobar, Rogelio Roque and Mavy H. Rodriguez

Mosquito-borne diseases are reported nowadays as re-emerging diseases, thus opening new vistas of research and plunging once more into chaos, altering what appeared to be a clear cut situation some years ago. For many years it has been believed, that the best way to interrupt malaria transmission is to target adult female mosquitoes with chemical insecticides. The integration of mosquito larval control with current interventions of malaria control program could be an important alternative to deal with resistance as well. Our objective was to operationalize the use of biological larvicides Griselesf (*B. sphaericus* 2362) and Bactivec (*B. thuringiensis* serotype H-14) as an alternative for malaria vector control to strengthen community participation in malaria control and malaria preventive interventions in Zambia, and to set up a system for monitoring and evaluation to be implemented. We conclude that larviciding using biological alternatives has been operationalized and has reached community ownership. Above 66 % malaria burden reduction was indeed achieved and properly documented, and the cost was USD 0.70 per inhabitant benefit when aerial and manual spraying had been combined. Other added values like the amount of people trained and the reduction of other mosquitoes of medical and nuisance importance were also reported.

### 166 The role of biolarvicides and community participation for *Aedes aegypti* control in South America

Mavy H. Rodriguez, mavygaby05@yahoo.com, Juan Beritán, Jorge L. Mendoza, Reynaldo G. Vergara, Uranis Filho, Efrain Beltrán, Dagoberto Enriquez, Laura Figueroa and Yalina Chang

Vector control activities play an important role in avoiding the emergence and/or re-emergence of dengue. Larval control is a preventive and effective strategy to control *Aedes aegypti* populations. The use of biological larvicides based on *Bacillus thuringiensis israelensis* (*Bti*) has proven its efficacy in the control of *Ae. aegypti* in different endemic countries. The purpose of this work is to show a summary of results obtained during the use of indoor application of *Bti* in an aqueous concentrated formulation jointly with community participation in some countries of South America. Urban and suburban areas of Brazil, Guatemala, Cuba, and Ecuador were treated, and we compared the Aedic Index between control areas (temephos and granular *Bti* application) and areas treated only with aqueous *Bti*. The dose used for biolarvicide application was 1 ml/50 liters of water and the treatment frequency ranged from 7 days to 1 month. The reduction percentage of the Aedic Index oscillated between 85 to 99% in the strictly aqueous *Bti* treated areas and showed a similar behavior in the above-mentioned countries. The innocuousness, specificity, and lack of toxicity of this formulation allows integrating the community in an active role in the treatment of the useful recipients, maintain vector densities at levels that do not represent epidemiologic risk

### 167 Evaluation of Mosquiron 0.12P for the control of mosquito larvae in Michigan

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To reduce the risk of mosquitoes developing resistance to pesticides and to ensure the most effective control possible, new chemical pesticides must be developed and evaluated. Mosquiron® is a controlled-release formulation of the insect growth regulator novaluron developed for the control of mosquito larvae. This material demonstrated 100% inhibition of adult emergence in earlier field trials. We investigated the effectiveness of Mosquiron 0.12P for control of mosquito larvae in container habitat in Michigan. One application provided effective control for the entire mosquito season.

### 168 Control efficacy of three pupicides in southern California catch basins

Gerald Chuzel, gchuzel@cvmvcd.org and Jennifer Henke

The Coachella Valley Mosquito and Vector Control District examined the effectiveness of 3 products labeled as pupicides. Agnique MMF, an ethoxylated alcohol, has been the product of choice for CVMVCD. This product was compared to Kontrol Larvicide and CocoBear, 2 mineral oil-based products. The study examined how the products worked in 21 catch basins using both lab-reared mosquitoes and field populations. Lab-reared mosquitoes were introduced using modified BioQuip® “mosquito breeders” and were replaced at each visit. Field populations were examined using a 2-dip method with a standard dipper. Water parameters (temperature, pH, conductivity, and dissolved oxygen) were measured at each visit. Results showed minimal larval populations at all sites 3- and 7-days post-treatment. Nearly half of the catch basins were still under control at 14-days post-treatment. No significant differences were seen between the 3 control products. No significant impacts to water quality were noted, and some sites continued to have small numbers of rat-tailed maggots throughout the trial.

### 169 Operational results of acoustic larvicide® in Asia, including Taiwan and Singapore

Herbert Nyberg, sales@newmountain.com

It has been discovered that acoustic larvicide® is the environmentally friendly alternative to eliminate mosquitoes at the larval stage. Larvasonic™ technology was investigated for use in water containers in Taiwan and Singapore with a Storm Drain Unit SD2001 machine. In Asia and many other parts of the world, rooftop mounted tanks are used to store and

pressurize the primary drinking and bathing water for residential housing units. These water sources attract female mosquitoes as primary oviposition sites. This presentation discusses in detail the results of research to combat *Aedes* in containers, as well as the development of new equipment for a permanent rooftop application. Ultrasonically-treated water is free of chemicals and contamination and can be recycled for human consumption.

#### 170 Larviciding success in a small district

Jennifer L. Mullins, jmulins548@hotmail.com

The Leavenworth Mosquito Control District encompasses about 4 square miles and was created in 2002 to control the large number of nuisance mosquitoes (*Aedes*) resulting from floodplain water and snowmelt ponds, as well as to reduce the possibility of West Nile virus transmission from *Culex* spp. Our mandate was to control mosquitoes through larviciding in so far as possible. We monitored the success of our operations through EVS traps. In our 1st and 4th year of operations we had high numbers (over 500 per trap) of nuisance mosquitoes and arranged for aerial spray contracts. In the rest of the years, adulticiding was unnecessary because of our success in locating and treating larval habitat sites. 2010 and 2013 were particularly successful years (aided by a moderate snowpack) in which we were able to reduce mosquito numbers to the point at which testing for West Nile virus was unnecessary (under 10 *Culex*). In 2010, the maximum number of mosquitoes caught in a trap was 12 (at 3 trap sites) and the maximum *Culex* caught was 7. In 2013, the maximum number of mosquitoes caught in a trap was 6 (1 site) and the maximum number of *Culex* caught was 4. My presentation will detail how I achieved this high degree of success through all resources available including public involvement.

### Education

#### 171 "Vector Inspectors." A novel program targeting schools in an area infested with *Aedes albopictus*

Kelly M. Middleton, kmiddleton@sgvmosquito.org, Carol Anne Hagele and Teresa E. Sorvillo

An infestation of *Aedes albopictus* was identified in the San Gabriel Valley (Los Angeles County, CA), in the fall of 2011 and presented numerous challenges. We needed to rapidly identify infested properties and conduct targeted outreach despite logistical and cultural challenges. In 2012, the District launched a unique elementary education program to: convey key public health information to families through school-aged children, motivate families to reduce sources of mosquitoes on their properties, and procure larval samples (for identification) from properties in the target area. We reached 16 schools and more than 7,500 students with this program, which successfully identified new properties infested with *Ae. albopictus* and reduced larval populations of all mosquitoes at participating households to levels below that detected during routine inspections in other areas. The program was modified in fall of 2013 making it available to individual classrooms, scout groups, and families. This paper highlights the use of Machinima<sup>SM</sup> animation as an education tool and presents the results of the Vector Inspector Citizen Science Projects.

#### 172 SPLAT (Students & Professionals All Learning Together)

Cynthia J. Mulla, cindymulla@comcast.net

The presentation will include a video consisting of interviews with administrators, teachers (from Pre-K through middle school) and students all reviewing the importance of a mosquito educational program. In addition, media professionals encouraging other mosquito control districts to build a cooperative working relationship with their local media outlets in order to get their timely public health messages broadcast to the public.

#### 173 Fleet vehicle management and sustainability

Lucas Griemann, ljgriemann@mmcd.org

This presentation will cover aspects of what the Minnesota Metropolitan Mosquito Control District does to manage their fleet of vehicles and what we are currently working on to better make decisions about vehicles we will purchase in the future to meet our sustainability goals.

### Measurement Techniques for Field Sampling Aerosols I

#### 174 Understanding aerosol partitioning and other important factors when sampling aerosols during open field trials

Mark Latham, manateemcd@aol.com

The use of field-deployed aerosol sampling devices in adulticiding spray trials is becoming more common as it provides a physical measure of the observed biological effect in caged mosquito bioassays or wild mosquito population reductions. These devices can be used both for measuring the aerosol cloud emitted at the nozzle ("droplet size calibration") and the fraction of the spray passing through the target area (spray "flux" or "transport"), often 100s or 1000s of feet downwind from the point of application. However, individuals reporting the results must understand how the sampled aerosol cloud in the target area differs from that emitted at the nozzle, how prevailing meteorology affects the sampling of the aerosol cloud, and what other factors need to be taken into consideration. The longer the aerosol cloud stays airborne, the greater the probability that the larger droplets will sediment out or be captured by natural filtering elements. Thus, the measured airborne fraction of the aerosol cloud gets progressively smaller the further downwind it is sampled, assuming that the selected sampling device is capable of effectively collecting small droplets. The volatility of the spray formulation is another important consideration when analyzing the collections from these sampling devices.

### 175 Measuring spray droplet in flight

Wesley C. Hoffmann, [clint.hoffmann@ars.usda.gov](mailto:clint.hoffmann@ars.usda.gov) and Bradley K. Fritz

The size of spray droplets during an adult mosquito control spray has a significant effect on the success or failure of the application. Laser diffraction instruments, hot wire anemometers, and glass slides are used by researchers and operational personnel to measure spray droplets. While each of these methods provides a measure of the droplet spectrum created by a given application method or piece of equipment, the results may not always agree as each method samples the sprays differently. Though the methods may not agree, each has distinct advantages and disadvantages that should be considered. Each of these methods will be discussed, as well as, how each method compares to the others.

### 176 Field calibration: measuring the droplets size distribution

Mike Reynolds, [MReynolds@LeaTeam.com](mailto:MReynolds@LeaTeam.com)

It is essential that an operator and researcher alike know what droplet size distribution they are applying. The capacity of the atomization device is generally determined in a laboratory setting by the manufacturer, but to ensure that the equipment is operating correctly, field calibration is necessary. This presentation discusses the different methods available to calibrate your machinery. In addition, this presentation will describe the methods used to calculate the descriptive statistics of the droplets size distribution.

### 177 The role of biological measures for characterizing aerosol distribution

Jane A. Bonds, [jasbonds@gmail.com](mailto:jasbonds@gmail.com)

To test the efficacy of a pesticide application, one can physically characterize the spray concentrations, but most important is a measure of biological response. Did the application work? Depending on the type of experiment, different methods can be employed. Each tool has its advantages and disadvantages. This presentation describes the options available, defining the associated bias with each method.

### 178 Analytical methods for determining active ingredients in horizontal and vertical samplers after ULV applications

Jing Zhai, [jingzhai@eurofins.com](mailto:jingzhai@eurofins.com)

Fluorimetry, HPLC and LC/MS/MS are common analytical methods for determining an active ingredient in a mosquito ULV application study. Application rates and spray equipment have a significant impact on the levels of active ingredient in horizontal and vertical samplers downwind from a spray path. The paper discusses importance for using a proper analytical method for measuring ultra-low levels of active ingredients.

## Measurement Techniques for Field Sampling Aerosols II

### 179 Volumetric sampling of spray flux

Bradley K. Fritz, [brad.fritz@ars.usda.gov](mailto:brad.fritz@ars.usda.gov) and Muhammad Farooq

Spray sampling can be conducted using a variety of methods, all of which have unique operational characteristics, including the sampling mechanics and the efficiency with which droplets are captured. Volumetric sampling, unlike other sampling methods, actively pulls in ambient air, which is then sampled for spray concentration. Volumetric sampling is typically a more expensive method and is used when human exposure (inhalation) is a concern. Unlike passive sampling devices, or even active samplers like rotorods or rotary impactors, volumetric sampling methods must be adjusted based on ambient airflow so that isokinetic (ambient air velocity equals air velocity entering sampler) sampling conditions are achieved. Like other sampling methods, volumetric samplers also have inherent inefficiencies in sampling and spray material presented to it that should be understood before using collected data for application or policy decision making. The advantages and disadvantages of volumetric sampling systems will be discussed.

### 180 Sampling airborne spray concentrations

Bradley K. Fritz, [brad.fritz@ars.usda.gov](mailto:brad.fritz@ars.usda.gov)

Understanding the amount and location of applied spray that is airborne at locations downwind of a spray site is critical for agricultural and human health applications. Accurately measuring the spray that remains suspended can provide a greater understanding with regard to adverse effects and efficacy. Measuring airborne spray is typically done using either active or passive spray samplers, both of which offer advantages and disadvantages in the field. Passive spray samplers, while not typically as efficient in collecting smaller droplets as active samplers, are much easier to understand with regard to collection efficiency and offer the ability to sample across a wider swath. Having knowledge of the collection efficiency allows for correcting to actual concentrations, which potentially allows for mass balance across the spray system. Active samplers offer a more efficient collector but are also more difficult to determine collection efficiency for as a result of the interaction of the sampler, spray characteristics and ambient wind conditions. These samplers also tend to be "spot" samplers covering only a narrowly defined point in the downstream spray swath. To overcome this, more samplers can be deployed, but at greater costs. There is no "perfect" sampler, but by considering the performance characteristics and limitations of the samplers available and balancing them against the needs of a given study, an optimized data set can be obtained.

**181 Ground deposition measures: the primary measure of non-target effect**

Jane A. Bonds, jasbonds@gmail.com

Ground deposition sampling is extremely important considering that the Environmental Protection Agency considers this as a measure of non-target effect. The physical characterization is typically conducted using filter papers as either a measure of pesticide or tracer concentration. The EPA to date prefers that direct measures of pesticide concentration be taken over tracers. Another important option is a biological measure of non-target effect. One of the EPA's primary concerns is pesticide deposition to water bodies. Pesticide loads can be measured in water but any uncontrolled measure is subject to misinterpretation. This presentation will discuss where and how each option is best integrated in to an experimental design.

**Poster Abstracts****Adult Control****P-01 Integrated control of *Aedes aegypti* in Iguala, Guerrero, Mexico**

Cipriano Gutiérrez Castro, cipriano\_dr\_saludgro@hotmail.com, Felipe Dzul-Manzanilla, Leonardo López-Damián, José Ibarra-López, Wilbert Bibiano Marín, Gustavo Sánchez-Tejeda, Juan I. Arredondo-Jiménez and Pablo Manrique-Saide

We studied the field-efficacy of integrated dengue vector control (IVC) with the 3 main activities performed by the dengue control programs (*e.g.*, larval control, indoor spraying, and ground vehicle-mounted ULV spraying) on the number of indoor-*Aedes aegypti* females in residential premises of Iguala, Guerrero, Mexico. We evaluated and compared the individual application of each activity and when the 3 activities were applied jointly and synchronized. Indoor-adult mosquito resting collections were conducted using CDC backpack aspirators 24 h before and 24 h after the interventions and followed weekly. If applied separately, only indoor spraying and ground vehicle-mounted ULV spraying had a protective effect on the abundance of *Ae. aegypti* females. IVC with all the activities synchronized definitely increases the effect on the abundance of indoor dengue vectors with 70%-100% reductions 24 h after, 79%-90% after 1 wk and 16%-36% after 5 wk.

**P-02 Entomological evaluation of ground vehicle-mounted ULV spraying on indoor female *Aedes aegypti* in Acapulco, Guerrero, Mexico**

Felipe Dzul-Manzanilla, fdzul@me.com, Cipriano Gutiérrez Castro, Leonardo López-Damián, José Ibarra-López, Andrés Martini Jaimez, Rodolfo Gutiérrez-Ortiz, Wilbert Bibiano Marín, Azael Che-Mendoza, Gustavo Sánchez-Tejeda, Juan I. Arredondo-Jiménez and Pablo Manrique-Saide

Ground vehicle-mounted ULV spraying is part of the portfolio of dengue prevention and control programs in Mexico. This study evaluated the field-efficacy of ULV space spraying on the number of indoor-collected female *Aedes aegypti* using 3 different application schemes: 1) 3 applications on consecutive days; 2) 3 applications every 2 days; and 3) 3 applications with 1 wk interval between each application. Indoor-adult mosquito resting collections were conducted using CDC backpack aspirators in residential areas of the city of Acapulco. All the schemes reduced ca 60% of both females and engorged-females immediately after 24 h. However, the application every 2 days had a larger and longer effect (2 wk) on the dengue vector densities inside of the houses, in comparison to the other schemes which only persisted for 1 wk. When this scheme of application is performed during sunrise and sunset, the efficacy increases and the number of indoor resting *Ae. aegypti* females is reduced by 96% after 24 h. Results of this study indicate that ground vehicle-mounted ULV spraying is effective on reducing dengue vectors, especially if applied twice a day and with 2 day intervals.

**Behavior/Biology****P-03 Molecular sensory networks in the maxillary palps of the yellow-fever mosquito, *Aedes aegypti***

Jonathan D. Bohbot, jonathan.bohbot@ars.usda.gov and Joseph C. Dickens

Female *Aedes aegypti* mosquitoes are obligate blood-feeding insects and vectors of pathogens that cause dengue, yellow fever, and a number of other diseases. Blood-feeding by female mosquitoes concludes a chain of multisensory events guiding the hungry mosquito to its host from a distance. Sensory receptors involved in this behavior include the compound eyes and chemoreceptors located primarily on the antennae and maxillary palps. In this study, we used modern sequencing techniques to generate a snapshot of the genes expressed in the physiologically-mature maxillary palp of female *Ae. aegypti*. We show that the genes expressed in the palps encode proteins involved in not only chemosensory reception, but also signal transduction and neuromodulation. Moreover, we identified candidate transcripts concerned with mechano- and thermosensation. While this survey lays the groundwork to explore the molecular sensory networks of this important sensory appendage in mosquitoes, the identification of genes involved in thermosensation provides new molecular targets for the development of drugs aimed at disrupting the behavior of this medically important insect.

**P-04 Characterization of gustatory receptor expression in labella and tarsi of the yellow-fever mosquito, *Aedes aegypti***  
Jackson T. Sparks, jackson.sparks@ars.usda.gov and Joseph C. Dickens

The yellow-fever mosquito, *Aedes aegypti*, infects a growing number of people every year with dengue, yellow fever, and chikungunya viruses. Contact chemoreception in mosquitoes influences a number of behaviors including host-selection, oviposition and feeding, as well as mediating, at least in part, responses to insect repellents. While these behaviors are in many instances well documented, the molecular mechanisms mediating them are not well understood. Here, we report the results of sequencing total messenger RNA in the labella and tarsi of both male and female *Ae. aegypti* to reveal gustatory receptor (GR) gene expression profiles in these major gustatory appendages. Gene expression levels in each tissue were secondarily verified by RT-qPCR. We discuss potential functions for the GRs revealed here by considering homologous GRs in other insects. Specific GRs may provide suitable molecular targets for modification of gustatory-mediated behaviors in this important disease vector.

**P-05 First record of *Aedes (Ochlerotatus) stimulans* from Louisiana**

Charles Sither, cbsither@gmail.com, Bruce Harrison, Britannia Bintz, Mark Wilson, Monty Graham, Jung Kim, Kevin A. Caillouet and Brian Byrd

The first confirmed record of *Aedes (Ochlerotatus) stimulans* for Louisiana was made in St. Tammany Parish, LA. A single adult female, identified by microscopic and molecular methods, was collected by a large-bore aspirator in March 2011. The results of scanning electron microscopic and COI DNA analyses are described. Morphological features are reviewed in comparison to more commonly collected mosquitoes in Louisiana. Typically, *Aedes stimulans* is a univoltine species whose larvae first appear in late winter and early spring in northern forested vernal pools. Therefore, its rarity in southern states may be explained (in part) by appearing at low abundance during "off season" surveillance periods for many mosquito control districts. An additional couplet is proposed for the *Keys to the Medically Important Mosquitoes of Louisiana* (Fox and Wesson, 2005) to assist local mosquito control personnel.

**P-06 A comparison of timing of host-seeking activity among three mosquito species in a Northern California rice field**  
Mary Sorensen, marys@placermosquito.org and Angella Falco

Two programmable traps with carbon dioxide attractant were set multiple times per week for 2 mo in summer 2013, allowing each mosquito collection to be separated into 1-h intervals from 6:00 p.m. to 7:00 a.m. Temperature and humidity were recorded at the site in 1-min intervals. The majority of mosquitoes captured were *Culex tarsalis*; however, enough *Cx. pipiens* and *Anopheles freeborni* were collected to analyze flight activity in relation to time and temperature as well as to compare activity of the 3 species.

**P-07 Comparative estimates of density and species diversity in adult mosquito populations landing on a human subject and captured using light and suction traps**

Donald R. Barnard, don.barnard@ars.usda.gov

The comparative responses of 21 species of mosquitoes to light traps (LT) and suction traps (ST) and captured using the human landing collection method (HL) varied in accordance with collection technique. Data analyses for most species revealed a significant interaction between collection method and collection interval (time of day collections were made). In one example, estimates of mosquito density (during the 60 min immediately following sunset) ranged from 0 (HL), 0.017 (LT), and 0.018 (ST) females per minute for *Culiseta melanura*, to 0.200 (HL), 0.054 (LT), and 0.040 (ST) females per minute for *Culex erraticus*, to 1.061 (HL), 2.499 (LT), and 0.298 (ST) females per minute for *Ochlerotatus infirmatus*. Indices of species diversity varied significantly according to collection method. Eight mosquito species (38%) were undetected by at least 1 of the 3 collection methods and 5 species (14%) were undetected by 2 of the collection methods. The results enabled development of species-specific numeric algorithms that can be used to relate mechanical trap-based estimates of adult mosquito density to the mosquito landing rate on a human being.

## Disease/Vector Studies

**P-08 House and school tests of insecticide treated nets for *Aedes aegypti* control in Acapulco and Merida, Mexico**

Azael Che-Mendoza, achemendoza\_vectores@hotmail.com, Guillermo Guillermo-May, Josué Herrera-Bojórquez, Mario Barrera-Pérez, Felipe Dzul-Manzanilla, Juan I. Arredondo-Jiménez, Hilary Ranson, Audrey Lenhart, Philip McCall, Axel Kroeger, Anuar Medina, Edgar U. Koyoc-Cardena, Yamili Contreras-Perera and Pablo Manrique-Saide

We report the first results of an intervention study with long-lasting insecticide treated nets (LLINs) fixed as mosquito screens on doors and windows of houses in the neighborhood Ciudad Renacimiento at Acapulco Guerrero and 10 neighborhoods of Merida Yucatan. Screens were made from Duranet Clarke® material (100% polyethylene non-flammable, 145 ± 5% Denier with a mesh of 132 complete holes/in.<sup>2</sup> / minimum 20 complete holes per cm<sup>2</sup> and insecticide content of 0.55% w.w ± 15% alphacypermethrin). Frames were made of aluminum by a local small business from the same locality. The effect of the interventions was determined by comparing pre-and post-intervention measures of the presence and abundance of indoor *Aedes aegypti* using modified CDC backpack aspirators between treated and untreated houses/areas of the same locality. Results showed a significant reduction on adult vector densities after protecting houses

with LLINs mosquito screens, particularly evident during the rainy season. In houses with mosquito screens the presence and abundance of *Aedes* females during the rainy season decreased >30% and >50% respectively, while in the control houses abundance increased.

**P-09 Passive baited sequential fly trap**

Robert L. Aldridge, Robert.Aldridge@ars.usda.gov, Seth C. Britch, Melissa Snelling, Gregory White, Arturo Gutierrez and Kenneth J. Linthicum

Sampling fly populations associated with human populations is needed to understand diel behavior and monitor population densities before and after control operations. Population control measures are dependent on the results of monitoring efforts as they may provide insight into fly behavior and the improve the likelihood of pesticide exposure. A novel trap is described for sequential collection of flies by means of an odorous bait and a passive design utilizing an inverted cone to exploit innate phototactic behavior. The sequential trap can be used to effectively monitor fly populations over a given range of time with at least 8 distinct collection periods. Trials have been conducted utilizing the novel sequential trap in hot arid desert environments with high fly densities of multiple species to monitor fly population density and diel behavior in conjunction with a standard passive baited trap. The novel sequential trap performed similarly to the standard passive baited trap in collection numbers and should be considered for use in diel rhythm and diel behavior studies of flies. Significant peaks in fly activity were detected at specific times of the day. This knowledge has permitted us to target control with pesticides.

**P-10 Detection of *Rickettsia* in brown dog ticks *Rhipicephalus sanguineus* collected from domestic dogs in Molas, Yucatan**

Edgar U. Koyoc-Cardena, edgarkoyoc@gmail.com, Beatriz Salceda-Sanchez, Roger I. Rodriguez-Vivas, Jorge C. Rodriguez-Buenfil, Daly Martinez-Ortiz, Azael Cohuo-Rodriguez and Pablo Manrique-Saide

Rickettsioses are caused by intracellular bacteria associated to arthropod vectors such as fleas, lice, and ticks. Rocky Mountain spotted fever and the epidemic and murine typhus (*Rickettsia rickettsii*, *R. prowazekii* and *R. typhi*, respectively) are among the most important diseases in humans caused by these bacteria. Rickettsioses, particularly *R. rickettsii*, have been increasingly reported in northern Mexico, but also sporadic cases are reported from southern Mexico and Yucatan. We report the preliminary results of the molecular detection of *Rickettsia* in ticks collected from domestic dogs in the rural community of Molas, Yucatan, Mexico. We collected 506 *Rhipicephalus sanguineus* (the brown dog tick) from 150 dogs randomly sampled in the study site from February to June 2012. Molecular detection was performed with a standardized protocol at the Instituto de Diagnóstico y Referencia Epidemiológicos of the Mexican Ministry of Health (PCR with R17-22 and R17-500 primers to detect the 17kDa antigen). We found rickettsial DNA in 17% (25/146) of all ticks tested. As far as we know, this is the first report of *Rickettsia* in *R. sanguineus* in Yucatan. More studies are ongoing to identify the *Rickettsia* species.

**P-11 Detection of West Nile virus antibodies in mosquito blood meals**

Nicholas Panella, nap4@cdc.gov, Ginger Young and Nick Komar

Typically, detecting the prevalence of antibodies to a particular arbovirus involves conducting a lengthy avian or mammal serological survey, processing of the sera and a 3-7 day plaque reduction neutralization test (PRNT). These investigations place great demand on human and financial resources. Recently, a new method was described to replace the traditional PRNT by using rapid high-throughput procedures that combined serum amine labeling via biotinylation, contaminant removal, and microsphere-based immunoassays for antibodies to West Nile virus (WNV), St. Louis encephalitis virus and eastern equine encephalitis virus. Although this greatly reduces the time it takes to obtain results from serum samples, the collection of samples from host species remains the same. In this study, we evaluate the biotin microsphere immunoassay (MIA) for its efficacy to detect WNV-specific antibodies in blood obtained from the abdomens of engorged mosquitoes. Detecting WNV-reactive antibodies in mosquito blood meals can facilitate the monitoring of seroprevalence in avian populations and be useful for surveillance and ecological studies.

**P-12 Epidemiology of West Nile virus in Québec, 2002-2013: what happened in 2011-2013?**

Anne-Marie Lowe, anne-marie.lowe@inspq.qc.ca, Christian Back, Cécile Ferrouillet and Anne Fortin

West Nile virus (WNV) is now endemic in southern Québec (Canada). After 2002 and 2003 (number of human cases: 20 and 17), the annual number of detected human cases ranged between 1 and 5. It increased to 42 in 2011 and to 133 in 2012 (85 neurological cases, 5 deaths). In 2012, the majority of cases were in the Montreal area (n = 102, 77%) and of all the symptomatic cases documented that year, 69% developed a neurologic complication. The crude incidence of neurologic cases in Québec in 2012 was 0.83/100,000 persons, twice the incidence observed in Ontario (0.45 neurological cases/100,000 in 2012). The ecology of WNV is similar in Quebec and Ontario, with *Culex pipiens* and *Culex restuans* as major mosquito vectors. However, in 2006, it was hypothesized in Quebec that the 2002-2003 events were unlikely to repeat and all vector surveillance and preventive vector control operations were stopped, whereas they continued in Ontario. Based on the observations of 2011 and 2012, it appears that the WNV strategy needs revision in Quebec, and that preventive measures in Ontario might have reduced the WNV burden of human cases.

**P-13 Natural vertical transmission of Dengue-1 virus in *Aedes aegypti* populations from Acapulco, Mexico**

Norma Martínez, nedith@lycos.com, Maximina Cruz, Felipe Dzul-Manzanilla, Cipriano Gutiérrez Castro, Leonardo López-Damián, Andrés Martini Jaimez, Herón Huerta-Jiménez, Mauricio Vázquez-Pichardo, Gustavo Sánchez-Tejeda, Juan I. Arredondo-Jiménez, Guadalupe Ayora-Talavera and Pablo Manrique-Saide

We carried out virological surveillance of dengue virus (DENV) in *Aedes aegypti* populations from 47 neighborhoods of Acapulco during the rainy season of 2011 as an innovation of the entomological surveillance of the Mexican Dengue Control Program. A total of 4,146 *Ae. aegypti* adults (emerged from eggs, larvae and pupa and/or collected inside houses as part of routine activities of vector surveillance) were grouped into pools and processed using a standardized serotype-specific fourplex real-time reverse transcription polymerase chain reaction assay (CDC DENV-1-4 RT-PCR). Overall, 2 (0.9%) of 224 pools of *Ae. aegypti* adults (1 pool of adults emerged from field collected larvae and other pool of indoor

collected adults) were positive for DENV-1. Only male adults were positive. This is the first report of vertical and transovarial transmission of DENV-1 in field-caught *Ae. aegypti* from Mexico.

**P-14 Cemeteries are effective sites for LACv and vector surveillance in Appalachia**

Rebecca Trout Fryxell, RFryxell@utk.edu, Kimberly Freyman, Armando Ulloa, Brian Hendricks, Dave Paulsen and Abelardo Moncayo

The mosquito-borne disease La Crosse encephalitis (LAC) is the leading cause of arboviral disease among children in the United States. Unfortunately, the Appalachian region now has the highest incidence risk in the nation and almost 75% of all US cases are reported here (Haddow and Odai 2009). In 2012, 9 pediatric cases of La Crosse encephalitis occurred in eastern Tennessee, including 1 death. While *Aedes (Ochlerotatus) triseriatus* has been the historical vector, *Ae. albopictus* and *Ae. (Oc.) japonicus* are 2 invasive species that may be important accessory vectors. In an attempt to detect LACv in active mosquito populations, our objective was to determine if cemeteries were effective sites for monitoring LACv and the vector population; consequently, we conducted an in-depth vector ecology study centered around the 2012 fatal case. Briefly, 38 cemeteries were selected within 10 radial mi of the fatal case. At each cemetery, 4 ovitraps baited with water and seed germination paper (egg paper) were placed at the 4 cardinal directions. Egg papers and water were replaced weekly, from September 5 - October 3, 2012, this yielded a total of 760 egg papers. Recovered egg papers (99.3%) were brought back to the laboratory where eggs hatched and adults emerged. Thus far, we have successfully recovered all 3 vector species and identified 4 positive pools of *Ae. (Oc.) triseriatus*. This preliminary data indicates cemeteries are effective sites for of LACv and vector populations.

**P-15 Use of risk models to predict human West Nile virus risk in Manitoba, Canada**

Richard S. Baydack, Richard.Baydack@gov.mb.ca, Robert Wang, Philip Curry and Scott Graham-Derham

Two mathematical models were developed as tools to measure and predict weekly West Nile virus (WNV) transmission risk in southern Manitoba. The first model, a multivariate logistic regression, was used to predict the probability that at least 1 WNV-neurological syndrome case would occur during a specified week. The second model, a multivariate Poisson regression, was used to predict the number of WNV-neurological syndrome cases that may occur during a specified week. Both models utilized environmental factors [degree days (base 14.3°C) and/or precipitation] and mosquito surveillance indicators (average counts of *Culex tarsalis*, proportion of positive pools) as potential predictive variables. Predicted outcomes from both models were compared with actual WNV-neurological exposure and case data from 2003 to 2013. Preliminary data suggests that the Poisson model in particular provided reliable and accurate measures of risk when compared to the actual situation. In addition, the Poisson model provided an early warning signal of the severity of the seasonal risk, particularly in higher activity years. Use of these types of models will aid public health officials in the assessment and communication of the weekly WNV risk during the course of a West Nile season.

## Equipment

**P-16 *Culex pipiens quinquefasciatus* collection efficiency differences among gravid trap types**

Kevin A. Caillouet, caillouet@stpmad.org and Banugopan Kesavaraju

Efficient collection of primary West Nile virus vectors *Culex pipiens pipiens* and *Cx. pipiens quinquefasciatus* is required for sensitive pathogen surveillance. A prior evaluation in Salt Lake City, Utah of 3 gravid trap types concluded that the Frommer updraft gravid trap collected significantly more *Cx. pipiens pipiens* mosquitoes than the CDC gravid trap and the improved Reiter gravid trap. We evaluated the efficacy of the CDC gravid trap and the Frommer updraft gravid trap to collect *Cx. pipiens quinquefasciatus* mosquitoes in St. Tammany Parish, LA. In the laboratory, we evaluated wind speed differences among the traps using a standardized platform and anemometer. The wind speed generated by the Frommer updraft gravid trap was nearly half the speed of the CDC gravid trap. In a field trial, the CDC gravid trap caught significantly more *Cx. pipiens quinquefasciatus* mosquitoes than the Frommer updraft Gravid trap. Collection differences among the 2 traps are likely the result of reduced suction caused by the series of screens designed to collect mosquitoes prior to passing through the fan in the Frommer updraft gravid trap. We hypothesize that flight behavioral differences may account for observed collection differences between the mosquito species.

## Genetics

### P-17 Differential identification of *Aedes triseriatus* (Say) and *Aedes hendersoni* Cockerell (Diptera: Culicidae) by a novel duplex PCR assay

Robert Wilson, rdwilson2@catamount.wcu.edu, Robin Harrison, Michael Riles, Gideon Wasserberg and Brian Byrd

*Aedes triseriatus* (Say) is the principle vector of La Crosse virus (LACv), which is the most common cause of pediatric arboviral encephalitis in North America. Here we report a novel species-specific polymerase chain reaction (PCR) assay that differentially identifies *Ae. triseriatus* and *Ae. hendersoni* Cockerell. Because these 2 sibling species differ in their abilities to transmit LACv, accurate identification is critical for surveillance, research, and control programs. This duplex assay can detect the presence of both species in a single PCR reaction and is therefore simpler and faster than previously reported methods. The results of field studies where this assay was used to determine the species identification are presented.

## GIS/GPS

### P-18 Implementation and operation of a new mobile GIS database tool we named MobileVC

Dan Damian, DDamian@mail.maricopa.gov, Kirk Smith and John Townsend

The operation and workflows for MobileVC will be demonstrated. We summarize the collaborative efforts used to capture, store, and analyze field collected surveillance data across Maricopa County, Arizona. Together as part of 5 functional groups we have successfully centralized environmental health spatial and tabular information into an enterprise SDE database.

### P-19 Application of geographic information system technology in vector surveillance of West Nile virus, Mississippi 2007-2012

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*Culex quinquefasciatus* is the primary vector responsible for human West Nile virus (WNV) transmission in Mississippi. It is the responsibility of local municipalities to implement vector control strategies, these activities should be driven by vector surveillance indicators. Using geographic information system technology to spatially display vector surveillance data may serve as a valuable resource in providing this guidance. Since 2007, the Mississippi State Department of Health has been involved in WNV testing of mosquitoes collected from targeted hot spots and random fixed locations during peak WNV transmission months, May-October. Between 2007 and 2012, 1,248 gravid traps were set in 67 cities throughout the state. Traditionally this data has been recorded in paper-based log books. To better utilize this information for surveillance and response efforts, these data were converted into an electronic format, geocoded, and exported to ArcGIS 10.1, ESRI for spatial analysis. This presentation will describe the past geographic distribution patterns and WNV infection rates of *Cx. quinquefasciatus* collected in Mississippi during routine vector surveillance for the years 2007-2012 which may be useful in identifying areas of high potential for human WNV transmission.

## Larval Control

### P-20 Control of *Aedes aegypti* in subsurface catch basins of Merida Yucatan with Natular® DT

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Street-located subsurface catch basins have been reported as important oviposition sites for *Aedes aegypti* L. in certain neighborhoods of Merida, Mexico. Particularly during the dry season, a certain type of catch basin (rectangular pits 146.25 cm long, 46.75 cm wide, 97.5 cm deep, covered with a steel grate and a vertical drain pipe connected to a dry well) retain water even in the absence of rainfall and, furthermore, can be frequently infested with *Ae. aegypti* immatures. A survey in 20 neighborhoods investigated the occurrence of *Ae. aegypti* infestation in these type of catch basins and evaluated the field-efficacy of the larvicide Natular® DT for the control of mosquitoes in positive-catch basins during a dry season. Positivity (presence of 3rd-4th instar larvae or pupae) for *Ae. aegypti* was measured by larval-funnel traps and followed every week from December 2012 to March 2013. Every catch basin found positive during the first and following weekly surveys was treated with Natular DT (1 - 1.35 g/tablet). In the baseline survey, a total of 280 catch basins were recorded of which 67% had water from which 19% was positive for *Ae. aegypti* immatures. The larvicide was highly effective against *Ae. aegypti*. Positivity in the treated catch basins was reduced in 100% after the application. The duration of complete absence of larvae and pupae of *Ae. aegypti* was confirmed after 8 wk and in some cases effective control was achieved after 3 months.

### P-21 Results of pilot test of biolarvicides against *Anopheles* vector in Nasarawa and Ogun states/Nigeria

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Nigeria contributed a quarter of the malaria burden in Africa. Despite continental efforts and enormous resources injected in malaria control, the scourge persists. Larviciding and source reduction have a major advantage in that they control



mosquitoes before they disperse and transmit disease. Our objective was to evaluate the field efficacy of the formulations of *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* strain 2362 against larvae of *Anopheles* mosquitoes in Nasarawa and Ogun States and to assess the residual effectiveness on the mortality of anopheline larvae under field conditions. Mosquito larvae identification and the total average number of the different mosquito-larvae present in 10 dips in each site were determined and recorded. Physical and chemical nature of the water was also noted, thus establishing baseline data. The results of the trial were computed. The method of spoon dips (WHO 1982) was used to collect the larvae. The 6 sites in Nasarawa State and 2 in Ogun State were visited after 24 h, 48 h and 72 h to determine the population of anopheline larvae present at each site to evaluate the field efficacy of the biolarvicides. At the 14th and the 30th day of the treatment, all the sites were again visited to assess the residual effectiveness of Bactivec® and Griselel® on the mortality of anopheline larvae under field conditions.

### **P-22 Use of polymers in the application of insect growth regulator, pyriproxyfen, against *Aedes albopictus***

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The insect growth regulator pyriproxyfen at a concentration of 0.01mg per 1L of water was embedded in different types and concentrations of polymers. The efficacy of these formulations in preventing adult *Aedes albopictus* emergence was tested in laboratory assays after treated surfaces were subjected to simulated aging and exposure to different concentrations of oak leaf infusion. There was no adult mosquito emergence on all newly applied treatments. After simulated aging, effectiveness of the formulations was significantly reduced in all treatments. The polymer treatment prevented a loss of efficacy of the embedded pyriproxyfen while treatments with oak leaf infusion accelerated the loss of effectiveness of pyriproxyfen. Findings from this study will be discussed in the context of integrated mosquito management against container mosquitoes.

### **P-23 Effects of catch basin gates on larval mosquito control**

Jennifer Henke, jhenke@cvmvcd.org and Gabriela Perezchica-Harvey

The Coachella Valley Mosquito and Vector Control District (CVMVCD) examined the catch basins that received multiple treatments in 2011. Four catch basins were selected to have gates installed. These 4 catch basins had been internally divided to capture debris and to prevent it from entering outflow pipes. The division allowed for urban runoff to pool behind the wall, creating an ideal larval mosquito habitat. To remedy the mosquito production, CVMVCD had 2 catch basins fitted with automatic retractable screens and 2 catch basins fitted with Baleen Bristle Screens. Sites were monitored in the spring of 2013 to determine how much debris was collected. Sites were also examined every 2 wk for 1-year post-installation for mosquito production, water depth, and water parameters (temperature, pH, conductivity, and dissolved oxygen). The daily deposition of debris into the catch basins was greatly reduced compared with the amount of material allowed to accumulate prior to installation. Mosquito production was compared with treatment history prior to installation of catch basin gates.

### **P-24 Larviciding success in a small district**

Jennifer L. Mullins, jlmullins548@hotmail.com

The Leavenworth Mosquito Control District encompasses about 4 square miles and was created in 2002 to control the large number of nuisance mosquitoes (*Aedes*) resulting from floodplain water and snowmelt ponds, as well as to reduce the possibility of West Nile virus transmission from *Culex* spp. Our mandate was to control mosquitoes through larviciding in so far as possible. We monitored the success of our operations through EVS traps. In our 1<sup>st</sup> and 4<sup>th</sup> year of operations we had high numbers (over 500 per trap) of nuisance mosquitoes and arranged for aerial spray contracts. In the following years, adulticiding was unnecessary as a result of our success in locating and treating larvicide sites. 2010 and 2013 were particularly successful years (aided by a moderate snowpack) in which we were able to reduce mosquito numbers to the point at which testing for West Nile virus was unnecessary (under 10 *Culex*). In 2010, the maximum number of mosquitoes caught in a trap was 12 (at 3 trap sites) and the maximum number of *Culex* caught was 7. In 2013 the maximum number of mosquitoes caught in a trap was 6 (1 site) and the maximum number of *Culex* caught was 4. My presentation will detail how the numbers changed over the years and how I achieved this high degree of success through all resources available including public involvement.

## Management

### **P-25 EID Atlantique, 40 years of mosquito control in salt marshes of western France**

Norbert Barbarit, contact@eidatlantique.eu, Jérôme de Maupeou and Sébastien Chouin

From the Morbihan to the Gironde county (West Atlantic coast), EID Atlantique is a French public agency allowed to control mosquitoes for more than 40 yr, providing the monitoring and control of harmful and potential vectors of disease mosquitoes. Major objectives of the agency are: entomological survey, diseases vectors and hurtful mosquitoes control, ecological expertise, public information and sensitization on health and veterinary issues. Twelve experts from the world of research have been gathered in a Scientific and Technical Council to advise EID Atlantique. Specially, environmental monitoring is conducted to assess unintended effects of mosquito control on non-target fauna.

Key figures: 60,000 acres of marshes and wetlands monitored; 182 municipalities; 56 permanent agents; 11 technical agencies; annual budget of 5 million Euros.

**P-26 Using One Health in vector control results in comprehensive solutions**

Nikos Gurfield, nikos.gurfield@sdcounty.ca.gov and Rebecca Lafreniere

One Health is the paradigm that animal, environmental and human health are inextricably linked and that long-term solutions to complex health problems require a coordinated response across these sectors. Mosquito and other vector control activities lend themselves readily to a One Health approach because they lie at the nexus of humans, animals (vectors) and the environment. They can have far ranging consequences beyond the traditional realm of vector control that can improve the lives and safety of communities. For example, in 2011, the detection of malaria in a person in San Diego, CA led to rapid mobilization of specialists from multiple sectors including public health, vector control, veterinary, hazardous materials, medical examiner, agriculture, and law enforcement. The integrated One Health response resulted in detection and control of malaria-competent mosquitoes, bird deaths, environmental contamination, law infractions, and likely averted additional casualties. The coordinated response of multiple different departments and agencies was possible because of prior collaborations, pre-established contacts, and good will. Adopting a One Health strategy in vector control leads to a better understanding of complex problems and more complete and long-lasting solutions.

## Operations

**P-27 West Nile virus in Minnesota: program adaptations over 10-plus years**

Diann Crane, dianncrane@mmcd.org, Sandy Brogren, Kirk Johnson and Carey LaMere

Since the Metropolitan Mosquito Control District's (MMCD) inception, the focus of surveillance and control was on *Aedes* spp. and vectors of La Crosse and western equine viruses. As West Nile virus (WNV) swept across the country after its introduction in 1999 in NY, MMCD staff developed a WNV program in anticipation of the virus's arrival in Minnesota, which occurred in 2002. The program included enhancements to established surveillance routines and testing mosquitoes and dead birds for WNV presence. Over the past 10+ years, several changes in surveillance and control strategies were necessary to target *Culex* vector species including mapping and treating catch basins and stormwater structures, establishing treatment thresholds for larval and adult *Culex*, and expanding adult surveillance to include gravid traps and CO<sub>2</sub> traps in the canopy. Some novel control approaches include using bicycle crews to treat catch basins in urban areas and working with municipalities to treat underground stormwater structures. We are also investigating the affects of temperature on vector populations and disease transmission, and implications for the future.

## Public Relations

**P-28 West Nile virus surveillance: a new way to share data in the province Québec**

Anne-Marie Lowe, anne-marie.lowe@inspq.qc.ca, Stéphanie Jodoin and Anne Fortin

The first West Nile virus (WNV) cases were diagnosed in the Canadian province of Québec in 2002. Since then, an integrated surveillance system (human, animal, mosquitoes) has been implemented. Following the flare up of human cases of 2011 and 2012 (respectively 42 and 133 cases), mosquito surveillance, which had been stopped in 2007, was revived for the 2013 season. A weekly surveillance bulletin has been developed to support public health authorities in decision making and to inform regional health boards of the evolution of WNV season. It covers Québec human surveillance (by region), mosquito surveillance data (abundance of *Culex pipiens/restuans* by region, number of WNV positive mosquito pools by region) and animal surveillance data (through passive surveillance of birds and other species). Integrated surveillance data is also provided, giving the evolution of human and animal cases in relation with the positive mosquito pools through time. Canadian and American surveillance data is presented, as well as information about the ongoing governmental intervention plan 2013-2015 against WNV. Bulletins have been published weekly online on the INSPQ WNV webpage since week CDC 29 (July 14th to 20th, 2013). The WNV webpage generated 1,242 visits between July 18 and September 9, 2013. Three bulletins (no.1, 2, and 4) generated the most visits (respectively 207, 146, and 808 visits). This innovative way to follow up on an emerging situation is so far efficient and useful.

**P-29 An introduction to infographics: using pictures to communicate complex ideas**

Ada Barros, adab@placermosquito.org

Many people are now turning to digital resources for their information. They are constantly seeking out and simultaneously being bombarded by new information. Whether or not the information is factual or useful is sometimes hard to determine. With a subject as complex and broad as mosquito and vector control, it is difficult to compete for attention in the digital realm, and we need a tool that will help convey complex information, quickly and simply, to adapt to shorter attention spans. Many templates are now available to create infographics, as well as many tutorials to create them from scratch. A brief summary of the available tools will be provided. A step-by-step guide to creating a simple infographic will also be provided. Once you have created your infographic, how and when should you use them? Most intuitively, infographics can be used on your social media platforms, especially on Facebook, where members of the public

## Abstracts

can comment on it and share it with their networks. Infographics can also be inserted into digital and printed fact sheets and brochures.

### P-30 Larviciding sites located through your calls to 548-5904

Jennifer L. Mullins, jlmullins548@hotmail.com

Our poster shows a variety of photos of small and large larviciding sites located through calls and complaints to the Leavenworth Mosquito Control District including secluded catch basins, tires, a small pond, a scummy swimming pool, and a large oxbow wetland with applicators treating it.

## Other

### P-31 Comparative field-testing of citronella and geraniol wristbands for personal protection

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Different types of wristbands were tested in urban Haifa, Israel, in an area of moderate mosquito biting pressure with *Culex pipiens* and *Aedes albopictus* as the 2 principal species. Testing was conducted in accordance with EPA recommended test guidelines and at the end of the product label claimed period of efficacy. We tested 6 products on forearms of volunteers; 3 with geraniol and 3 with citronella as the main active ingredients. Compared to unprotected controls, 2 of the geraniol wristbands and 1 of the citronella wristbands performed well: Snap-Band, Fasst Products with protection of 87%; Insekten-weg, Bio-Natur-84%; and Buzz-Barrier, Green Compass Products-78%. The other wristbands performed poorly with protection rates less than 50%: SuperBand, Evergreen Research, geraniol based- 47%; PIC Citronella Plus, PIC-Corp.-36%; and Simba Natural Mosquito Repellent Wristbands, Simba Taiwan citronella based- 21%.

### P-32 Household water storage and potential mosquito breeding sites in Brisbane, Queensland

Jonathan Darbro, Jonathan.Darbro@qimrberghofer.edu.au, Brendan Trewin, Cassie Jansen and Timothy Hurst

We carried out backyard surveys in Brisbane, Australia, to assess the presence/absence of exotic mosquitoes (*Aedes aegypti* and *Ae. Albopictus*) and to define factors that can predict mosquito production. Of 13,151 houses visited, 4,953 were surveyed. A total of 6,570 immature mosquitoes were collected from 54,481 containers. The dominant species were *Ae. notoscriptus* (62.1%) and *Culex quinquefasciatus* (36.3%). No *Ae. aegypti* or *Ae. albopictus* were found. Garden accoutrements (e.g., flowerpot bases, bird baths), discarded household items and water storage containers (excluding rainwater tanks), made up 79.5% of positive containers. Of 2,713 rainwater tanks recorded, 27 (1.0%) were positive. Both species were likely to be found in gully traps, water features, and plant pots/bases. *Aedes notoscriptus* was more likely also to be found in birdbaths and *Cx. quinquefasciatus* in fishponds and wheelbarrows. The Premise Condition Index was positively associated with *Ae. notoscriptus* positivity but not *Cx. quinquefasciatus*. We found no relationship between socioeconomic (SE) status and *Cx. quinquefasciatus* production. Rubbish and water storage containers were more likely to be positive for *Ae. notoscriptus* in low (deciles 1-5) SE premises than in high ones (deciles 8-9); building fixtures, natural habitats and garden accoutrements were more likely to be positive for *Ae. notoscriptus* in higher areas. The potential for monitoring and risk assessment of container-inhabiting mosquitoes will be discussed.

## Latin American

### P-33 Taxonomic distinctiveness and phylogenetic relationships for seven *Anopheles* (Diptera: Culicidae) species of the Arribalzagia Series from Colombia using multilocus data

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Morphologically closely related *Anopheles* mosquito species represent a challenge for vector biologists. In addition to being involved in malaria transmission, some species included in the Arribalzagia Series, subgenus *Anopheles*, are difficult to identify using morphological criteria alone; therefore, confirmation by molecular markers is required. Mitochondrial *COI* and nuclear ITS2 sequences were used to identify 7 morphospecies included in the Arribalzagia Series from Colombia and to evaluate their phylogenetic relationships. Average K2P mean intra- and interspecific distances were 1.64% and 9.8% for *COI*, and 1.2% and 48.8% for ITS2 respectively. There was no barcoding gap due to overlap of intra- and interspecific variation for either marker. However, phylogenetic trees based on NJ, ML and BI approaches with each marker supported discrete clades at 100% for each of *Anopheles punctimacula* s.s., *An. calderoni*, *An. malefactor*, *An. neomaculipalpus*, *An. apicimacula*, *An. mattogrossensis* and *An. peryassui*. A BI analysis of the concatenated dataset (*COI*+ITS2) recovered the same clades. Furthermore, all analyses and datasets except *COI* alone supported a larger clade composed of *Anopheles calderoni*, *An. punctimacula* and *An. malefactor*, congruent with the informally designated Punctimacula Group. Correctly identifying putative vector species can assist in designing targeted control methods.

**P-34 Detecting Cys1534 and Ile1016 mutations in the voltage gated sodium channel gene in *Aedes aegypti* from Mexico**

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In recent decades, pyrethroid insecticides have played a major role in the control of adult *Aedes aegypti* mosquitoes. However, the evolution of resistance to these and other insecticides in *Ae. aegypti* may compromise the effectiveness of vector control programs. The early detection of insecticide resistance mutations is important for informed decisions concerning insecticide rotation. We analyzed the frequency of 2 mutations in the voltage gated sodium channel gene (*para*) in *Ae. aegypti* through melting curve-PCR. We assessed the replacement mutation in codon 1534 that encodes a cysteine rather than a phenylalanine and confers resistance to permethrin. Also, we calculated the frequency of a non-synonymous mutation in codon 1016 that encodes an isoleucine rather than a valine. A total of 362 *Ae. aegypti* were collected from 7 sites in the state of Veracruz, Mexico, in 2012. The allele frequency for Cys1534 and Ile1016 was determined for each mosquito collection. Higher Cys1534 allele frequencies relative to Ile1016 were obtained in all collections sites. An average allele frequency of 0.96 for Cys1534 and 0.73 for Ile1016 indicates pyrethroid pressure impacts mosquito control in Veracruz. These results support the rotation to non-pyrethroids in future vector control.

**P-35 Phylogeography of *Anopheles (Nyssorhynchus) nuneztovari* in northern South America**

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*Anopheles nuneztovari* s.l. is a complex composed of at least 2 species, widely distributed in South America (SAM). We hypothesized that climate changes during the Last Glacial Maxima-LGM and the presence of the Andes could promote expansion and possibly diversification in *An. nuneztovari* in northern South America (NSA). The demographic history of *An. nuneztovari* from NSA was evaluated to infer evolutionary processes. *COI* sequences of *An. nuneztovari* from Colombia and from different regions of SAM downloaded from GenBank were analyzed for evolutionary relationships by Bayesian inference (BI) and median joining was used to generate a haplotype network. Recent population expansion was evaluated with Bayesian Skyline Plots-BSP. The NSA sequences formed a well supported clade that included *An. nuneztovari* specimens from Colombia, Venezuela and Bolivia, and a subclade consisting of the eastern populations. The BI results suggest the Andean Mountains as a possible barrier promoting diversification within this species. The BSP analysis revealed an expansion of all populations except the northeast/central Amazon Brazil clade. Expansion times varied in the different clades; the most recent expansion was estimated at 20,000 years ago for the NSA clade. These results may indicate a strong influence of the LGM on the NSA populations

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## AMCA AWARDS

### HONORARY MEMBERS

1937	Leland O. Howard (USDA)	1965	Arthur W. Lindquist (KS)	1991	Kenneth L. Knight (NC)
1938	C. C. Adams (NY)	1967	Fred L. Stutz (FL)	1994	Harold C. Chapman (LA)
1944	Thomas J. Headlee (NJ)	1970	Robert L. Vannote (NJ)		Lewis T. Nielsen (UT)
	William B. Herms (CA)		Richard W. Fay (USPHS)	1998	Eugene J. Gerberg (MD)
	J. A. LePrince (USPHS)	1971	Christian T. Williamson (NY)		Glen C. Collett (UT)
	Louis L. Williams, Jr. (USPHS)	1972	Alan R. Stone (MD)	1999	Donald R. Johnson (GA)
1948	Robert D. Glasgow (NY)		Edward S. Hathaway (LA)	2001	Fred W. Knapp (KY)
	Willard V. King (USDA)	1974	Theodore G. Raley (CA)	2003	E. John Beidler (FL)
1951	Lewis W. Hackett (CA)	1976	John A. Mulrennan, Sr. (FL)	2004	David A. Dame (FL)
	Robert Matheson (NY)	1979	Thomas D. Mulhern (CA)	2005	Donald J. Sutherland (NJ)
1955	Harold F. Gray (CA)		Austin W. Morrill, Jr. (CA)	2006	Martin S. Chomsky (NJ)
1958	Louis A. Stearns (DE)	1981	William R. Horsfall (IL)	2013	Judy Hansen (NJ)
1964	George H. Bradley (USPHS/USDA)	1983	Anthony W. A. Brown (WHO)		Henry Rupp (NJ)

### 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 per year.

1972	Maurice W. Provost (FL)	1982	Kenneth L. Knight (NC)	1997	Robert K. Washino (CA)
	William R. Horsfall (IL)		William C. Reeves (CA)	1998	John D. Edman (MA)
1973	Don M. Rees (UT)	1983	Harry D. Pratt (GA)	1999	Bruce F. Eldridge (CA)
	Thomas D. Mulhern (CA)		John A. Mulrennan, Sr. (FL)	2000	Judy A. Hansen (NJ)
1974	Anthony W. A. Brown (WHO)	1984	George T. Carmichael (LA)	2001	Gary G. Clark (USPHS)
	Donald L. Collins (NY)	1985	Norman G. Gratz (WHO)	2002	Lucas G. Terracina (LA)
1975	Daniel M. Jobbins (NJ)	1986	James R. Caton (CA)	2003	Robert J. Novak (IL)
	Arthur W. Lindquist (USDA)	1987	Jay E. Graham (UT)	2004	James D. Long (TX)
1976	Austin W. Morrill, Jr. (CA)	1988	Lewis T. Nielsen (UT)	2005	James W. Robinson (FL)
	Carroll N. Smith (USDA)	1989	Andrew J. Spielman (MA)	2006	John L. Clark Jr. (IL)
1978	James B. Kitzmiller (FL)	1990	Glen C. Collett (UT)	2007	E. John Beidler (FL)
	William D. Murray (CA)	1991	Harold C. Chapman (LA)	2008	David A. Dame (FL)
1979	Richard F. Peters (CA)	1992	D. Bruce Francy (CO)	2009	Dan Ariaz (NV)
1980	William E. Bickley (MD)	1993	Gilbert L. Challet (CA)		Gary Breeden (VA)
	John N. Belkin (CA)	1994	Ronald A. Ward (MD)	2010	Mir S. Mulla (CA)
1981	Stanley J. Carpenter (CA)	1995	T. Wayne Miller (FL)	2011	Dave Brown (CA)
	Roland E. Dorer (VA)	1996	Marshall Laird (New Zealand)	2012	Sammie L. Dickson (UT)
				2013	Wayne Crans (NJ)

### 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)	1979	Marco. E. C. Giglioli (BWI)	1995	Frederick W. Wagner (KY)
	Donald L. Collins (NY)	1980	James D. Gorman (FL)	1996	Donald J. Sutherland (NJ)
	Theodore G. Raley (CA)		Donald E. Weidhaas (FL)		Ronald A. Ward (MD)
1973	Francis P. Creadon (CA)		E. John Beidler (FL)	1997	Roger S. Nasci (CO)
	Vernon Conant (NJ)		Eugene J. Gerberg (MD)		Thomas J. Zavortink (CA)
	Austin W. Morrill, Jr. (CA)	1981	A. Ralph Barr (CA)	1998	James D. Long (TX)
1974	Leslie D. Beadle (USPHS)		Gilbert L. Challet (CA)	1999	Hilton B. Munns (CA)
	John H. Brawley (CA)		Edgar A. Smith (VA)	2000	Leroy J. Bohn (VA)
	John W. Kilpatrick (GA)	1982	Hugo A. Jamnback (NY)		Dreda McCreary (VA)
	T. Oscar Fultz (GA)		Donald R. Johnson (GA)	2001	Charles T. Palmisano (LA)
	Howard R. Greenfield (CA)		Harold D. Newsome (MI)	2002	Thomas G. Floore (FL)
	Paul J. Hunt (FL)		James V. Smith (GA)		Sherry McLaughlin (TX)
	William C. McDuffie (USDA)	1983	Richard F. Darsie (CO)	2003	Wayne L. Kramer (NE)
	Donald R. Johnson (GA)		Ronald A. Ward (DC)		John L. Clarke, Jr. (IL)
	Helen Sollers-Riedel (DC)	1984	Samuel G. Breeland (FL)	2004	Yadira N. Rangel (Venezuela)
1975	Lewis E. Fronk (UT)		Donald J. Sutherland (NJ)		James W. Robinson (FL)
	Joseph G. McWilliams (USN)	1985	John C. Kuschke (NJ)	2005	Major S. Dhillon (CA)
	Lewis J. Ogden (USPHS)		James R. Caton (CA)		William H. Meredith (DE)
	Rajindar M. Pal (WHO)	1986	C. Lamar Meek (LA)	2006	William J. Sames (WA)
	Kenneth D. Quarterman (USPHS)	1987	John C. Combs (CA)	2007	Henry R. Rupp (NJ)
	Herbert F. Schoof (USPHS)	1988	Chester G. Moore (CO)	2008	Allan Inman (CA)
1976	Robert A. Armstrong (MA)		Margaret Parsons (OH)		Manuel Lluberas (FL)
	Osmond P. Breland (TX)	1989	John S. Billodeaux (LA)	2009	Joe Conlon (FL)
	George B. Craig, Jr. (IN)		Edgar S. Bordes, Jr. (LA)	2010	Norbert Becker (Germany)
	Claude M. Gjullin (USDA)	1990	Richard D. Morton (WA)	2011	Harry Savage (CO)
	T. Wayne Miller (FL)		Lucas G. Terracina (LA)		L.A. Williams (SC)
1976	Donald J. Pletsch (Mexico)	1991	David A. Dame (FL)	2012	Lal S. Mian (CA)
	Glenn M. Stokes (LA)	1992	Jerry Mix (TX)		Edsel M. Fussell (FL)
	Luis M. Vargas (Mexico)	1993	William E. Hazeltine (CA)	2013	Kenneth J. Linthicum (FL)
1978	Richard C. Axtell (NC)	1994	Sally A. Wagner (MI)		

### 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)	1991	James D. Long (TX)	2002	Dennis Moore (FL)
	Leslie E. Fronk (UT)	1992	Charlie D. Morris (FL)		Henry R. Rupp (NJ)
	Jesse B. Leslie (NJ)	1993	Robert J. Novak (IL)	2003	James R. McNelly (NJ)
1981	Linda G. Raiche (CA)	1994	James W. Robinson (FL)		Robert Bonnett (MN)
	Margaret S. Slater (NY)		Dan L. Ariaz (NV)	2004	James R. Brown (FL)
1982	K. G. Nolan (NY)	1995	Sally Kuzenski (LA)	2005	Mark Newberg (IL)
	Charles F. Scheel (IL)	1996	Carl R. Tanner (IL)		Susan Maggy (CA)
1983	Coyle E. Knowles (NY)		Sammie L. Dickson (UT)	2006	Teung Chin
1984	Ray Treichler (DC)	1997	Charles T. Palmisano (LA)	2007	Karl Malamud-Roam (CA)
1985	Lawrence T. Cowper (USAID)		George J. Wichterman (FL)	2008	William H. Meredith (DE)
	Janice B. Wells (NY)	1998	Douglas B. Carlson (FL)	2009	Rep. Dennis Cardoza (CA)
1986	T. Oscar Fultz (GA)	1999	Charles Beesley (CA)	2010	Gordon Patterson (FL)
1987	Sharon A. Colvin (IL)		Donald R. Johnson (GA)		Gary Clark (FL)
1988	Daniel D. Sprenger (TX)	2000	Peter B. Ghormley (CA)		Yasmin Rubio-Palis (Venezuela)
1989	Fred C. Roberts (CA)		David A. Brown (CA)	2011	Angela Beehler (WA)
1990	Leonard E. Munsterman (IN)	2001	Donald Menard (LA)		Roxanne Connelly (FL)
			Joel Margalit (Israel)	2012	Truc Dever (CA)
				2013	Robert Peterson (MT)

## Awards and Officers

### 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)	1997	Christine J. Dahl (Sweden)
1982	Lloyd E. Rozeboom (IL)	1998	Ralph E. Harbach (UK)
1983	Kenneth L. Knight (NC)	1999	Yiau-Min Huang (DC)
1984	Thomas J. Zavortink (CA)	2000	Lewis T. Nielsen (UT)
1985	Stanley J. Carpenter (CA)	2001	John F. Reinert (FL)
1986	Elizabeth P. Marks & John Reid (Australia)	2002	Richard F. Darsie (FL)
1987	James B. Kitzmiller (FL)	2003	Richard C. Wilkerson (MD)
1988	Allan R Stone (MD)	2004	Kazuo Tanaka (Japan)
1989	Pedro Galindo (Panama)	2005	Ronald A. Ward (MD)
1990	Peter F. Mattingly (UK)	2006	William K. Reisen (CA)
1991	Jose P. Duret (Argentina)	2008	Maria-Anice Sallum (Brazil)
1992	Bruce A. Harrison (NC)	2010	Daniel Strickman (MD)
1993	Edward L. Peyton (DC)	2011	Rampa Rattanarithikul, Ph.D. (Thailand)
1994	Theodore H. G. Aitken (CT)	2012	Maureen Coetzee, Ph. D. (South Africa)
1995	Oswaldo P. Forattini (Brazil)	2013	John F. Anderson (CT)
1996	A. Ralph Barr (CA)		
	Michael W. Service (UK)		

### 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. Brown	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 & LaCrosse 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. Belkin
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. Knipling	Waldemar Klassen	Titan and Driving Force in Ecologically Selective Area-Wide Pest Management

**MEMORIAL LECTURE HONOREE & MEMORIAL LECTURER AWARD (continued)**

	<b>HONOREE</b>	<b>LECTURER</b>	<b>TOPIC</b>
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	Remembering Norman Gratz (1925-2005) – Doyen of Vector Control
2007	Norman G. Gratz	Graham B. White	
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	

**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.	2005	Wesley Rubio	San Diego State U.
1990	Andrea Brown	Peru State Coll.		Whitney Qualls*	Auburn University
1991	John Paul Mutebi	Notre Dame U.		Rebecca Trout*	University of Kentucky
1992	Rosmarie Kelly	U. Massachusetts	2006	Robert D. Anderson	University of Winnipeg
1993	Merry L. Holliday-Hanson	U. California, Davis		Linda O'Connor**	University of Delaware
1994	John E. Gimnig	U. California, Davis		Joshua R. Ogawa*	Oregon State University
	Alice Shaeffer*	U. Mainz, Germany		Matthew Eaton*	Concordia College
1995	Glen Scoles	Notre Dame U.		Linda M. Styer*	U. California, Davis
	Jittawadee Rocharoen*	U. California, Riverside	2007	Jennifer Armistead	University of Florida
1996	Esther Chow Schaeffer	U. Maryland		Robert D. Anderson*	University of Delaware
1997	Lynn Cooper	U. Maryland		Thomas M. Mascari*	Louisiana State U.
1998	C. Roxanne Rutledge	Louisiana State U.	2008	Jerome Schleier	Montana State University
	Emmalee Kennedy*	U. Illinois		Christopher Barker*	U. California, Davis
	Timothy Schaub*	U. Illinois		Lisa Reimer*	U. California, Davis
1999	Laura Harrington	U. Massachusetts	2009	Alexandra Chaskopoulou	University of Florida
	Adam S. Jones*	U. Massachusetts		Stephanie Larick*	University of Florida
	Hillary Reno*	U. Illinois	2010	Sarah Wheeler	University of California, Davis
2000	Jason L. Rasgon	U. California, Davis		Kimmy Mains*	University of Kentucky
	Hope Q. Liu*	Virginia Polytechnic		Holly Tuten*	Clemson University
2001	No competition		2011	Logan Minter	University of Kentucky
2002	Laura B. Goddard	U. California, Davis		Kristen Meckel-Parker*	San Diego County Vector Control
	Sharon L. Minnick*	U. California, Davis	2012	Jerome Schleier	Montana State University
	Margaret Sherriffs*	Yale U.		Elizabeth Andrews*	University of Kentucky
2003	Sarah Yaremych	U. Illinois		Jennifer Gordon*	University of Kentucky
	Laura Goddard*	U. California		Joseph Iberg*	University of Georgia
	Jason L. Rasgon*	U. California, Davis	2013	Brian Johnson	Rutgers University
2004	Gregory M. Williams	U. Delaware		Andrea Egizi	Rutgers University
	Stephen Aspen*	Colorado State U.		Brittany Nelms	UC Davis - CVEC
	Christian Kaufmann*	U. Zurich			

\* - Honorable Mention \*\* - First Runner Up

## AMCA Awards and Officers

### 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)	2003	Allen W. Wooldridge	2010	Peter Connelly (FL)
1998	William German (FL)	2004	John L. Clarke, Jr. (IL)	2011	David Sullivan (MT)
1999	Gary A. Mount (FL)	2005	Ernest Danka (IL)	2012	Stephanie Whitman (WY)
	Daniel F. Boyd (GA)	2006	Willie N. Cox (IL)	2013	Larry Erickson (IL)
	David W. Waldron	2007	Bob Bonnett (MN)		
	J. David Waldron (GA)	2009	Clarke Hudson (IL)		
2002	Robert E. Richard (TX)		Bill Strange (ID)		

### 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	2012	Mike Smith	Anastasia MCD, Florida
	Christopher Trapp	Multnomah County Vector Control, Oregon	2013	Arturo Gutierrez	Coachella Valley MVCD, California
2006	John Phelps	Mercer County, New Jersey		Michael Martinez	Coachella Valley MVCD, California
2008	Chris Frame	Cape May County, New Jersey		David Lopez	Greater Los Angeles County VCD, California
2009	Jason Craig Hardman	Salt Lake City MAD, Utah		Martin Serrano	Greater Los Angeles County VCD, California
2010	Jessica Fales	Midland County MC, Michigan			
	Gary Hillsdale	Metropolitan MCD, Minnesota			
	Elizabeth Vice	Buttle County MVCD, California			
2011	David Bruget	Kings MAD, California			
	Russell Eck	Washoe County Health District,			
	Phillip Henry	Butte County MVCD, California			
	Levi Zahn	Williston VCD, North Dakota			

## AMCA OFFICERS, EXECUTIVE DIRECTORS AND EDITORS

### AMCA PRESIDENTS

1935-1939	Thomas J. Headlee*	1965-1966	Anthony W. A. Brown	1989-1990	Judy A. Hansen
1939-1940	Christian T. Williams*	1966-1967	Jay E. Graham	1990-1991	Robert C. Sjogren
1940-1942	Louis A. Stearns*	1967-1968	Harry D. Pratt	1991-1992	Matthew Yates
1942-1944	Robert C. Botsford*	1968-1969	Thomas D. Mulhern	1992-1993	Cyrus R. Lesser
1944-1945	Robert L. Vannote	1969-1970	George T. Carmichael	1993-1994	John A. Mulrennan, Jr.
1945-1946	Perry W. Ruth	1970-1971	Albert W. Buzicky	1994-1995	Chester G. Moore
1946-1947	Harry H. Stage	1971-1972	Andrew J. Rogers	1995-1996	John D. Edman
1947-1949	H. Duke Peters	1972-1973	Glen C. Collett	1996-1997	Robert J. Novak
1949-1950	Harold F. Gray	1973-1974	Kenneth L. Knight	1997-1998	Gary G. Clark
1950-1951	Lester W. Smith	1974-1975	Robert M. Altman	1998-1999	Dan L. Ariaz
1951-1952	Don M. Rees	1975-1976	Harold C. Chapman	1999-2000	William J. Zawicki
1952-1953	Cecil R. Twinn	1976-1977	D. Bruce Francy	2000-2001	David A. Dame
1953-1954	Fred C. Bishopp	1977-1978	Lewis T. Nielsen	2001-2002	Sammie L. Dickson
1954-1955	Roland E. Dorer	1978-1979	Paul J. Hunt	2002-2003	David A. Brown
1955-1956	Richard F. Peters	1979-1980	Glen M. Stokes	2003-2004	Fred W. Knapp
1956-1957	Fred L. Stutz	1980-1981	Robert K. Washino	2004-2005	Roger S. Nasci
1957-1958	Arthur W. Lindquist	1981-1982	Claude H. Schmidt	2005-2006	William R. Opp
1958-1959	John M. Hirst	1982-1983	Richard C. Axtell	2006-2007	Joseph F. Sanzone
1959-1960	Archie D. Hess	1983-1984	Jimmy K. Olson	2007-2008	Gene R. Payne
1960-1961	Daniel M. Jobbins	1984-1985	Gilbert L. Challet	2008-2009	Major S. Dhillon
1961-1962	William E. Bickley	1985-1986	T. Oscar Fultz	2009-2010	Doug Carlson
1962-1963	Arthur W. Geib	1986-1987	Donald J. Sutherland	2010-2011	Janet McAllister
1963-1964	Don W. Micks	1987-1988	George B. Craig, Jr.	2011-2012	William H. Meredith
1964-1965	John A. Mulrennan, Sr.	1988-1989	Bruce F. Eldridge	2012-2013	Thomas R. Wilmot
				2013-2014	Roxanne Connelly

\* - Eastern Association of Mosquito Control Workers

**AMCA TREASURERS**

1935-1943	Thomas D. Mulhern *	1985-1986	Douglas C. White
1944-1950	Thomas D. Mulhern	1986-1988	C. Lamar Meek
1950-1953	Roland E. Dorer	1989-1994	John S. Billodeaux
1954-1964	Lester W. Smith	1994-2000	Charles T. Palmisano
1965-1979	William D. Murray	2000-2011	Allan D. Inman
1980-1985	James R. Caton	2011-present	Gary Hatch

\* - Eastern Association of Mosquito Control Workers

**SECRETARY, EXECUTIVE SECRETARY, EXECUTIVE DIRECTOR**

1935-1943	Thomas D. Mulhern*	Secretary	1985-1986	James R. Caton	Interim Executive Director
1944-1950	Thomas D. Mulhern	Secretary	1986-1991	Harold C. Chapman	Executive Director
1950-1952	Thomas D. Mulhern	Executive Secretary	1991	Lucas G. Terracina	Acting Executive Dir.
1953-1973	Theodore G. Raley	Executive Secretary	1992	Mark Vinsand	Executive Director
1973	Theodore G. Raley	Executive Director	1992-1993	Harold C. Chapman	Executive Director
1974-1978	Thomas D. Mulhern	Executive Director	1993-1994	Lucas G. Terracina	Acting Executive Dir.
1979-1980	William D. Murray	Executive Director	1994-1995	Robert T. Graham	Executive Director
1980-1985	Thomas D. Mulhern	Executive Director	2006-present	Sarah B. Gazi	Executive Director

\* - Eastern Association of Mosquito Control Workers

**BUSINESS MANAGER**

1995-1999	Pamela D. Touns	2001-2004	Martin. S. Chomsky
1999-2000	Marlene Comeaux	2004-2006	Sarah B. Gazi
2000-2001	Robertamarie Kiley		

**TECHNICAL ADVISOR**

2000-present	Joseph M. Conlon
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**EDITORS OF JOURNAL OF AMCA\***

1941	Edited by the Publications Committee, Lester W. Smith, Chair†	1981-1996	Ronald A. Ward
1942-1943	Edited by the Publications Committee, Ralph W. Vanderwerker, Chair‡	1996-1998	Robert K. Washino
1944	Edited by the Publications Committee, J. T. Hart, Chair	1999-2003	Bruce F. Eldridge
1944-1948	Robert D. Glasgow	2004-2006	Kenneth J. Linthicum
1949-1973	Donald L. Collins	2007-present	Lal S. Mian
1973-1981	William E. Bickley		

\* - *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	1992-1993	Lewis T. Nielsen & Ralph E. Harbach, co-editors
1979-1992	Lewis T. Nielsen	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





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