Oral Presentation Abstracts

Plenary Session

PL-1 Are We Superheroes Fighting Transformers?

Dan Strickman, dan.strickman@gatesfoundation.org

Mosquito abatement districts (MADs) successfully fight an array of mosquitoes, intelligently combining methods in response to data and based on good science. Considered objectively, MADs are real-life superheroes, accomplishing what individuals cannot imagine. In fact, probably few of our customers realize that they are being protected as MADs go about their business in the background. It has taken years of technological development of machines, chemicals, and information systems to get where we are today, not to mention the continual cycles of training at all skill levels. The MAD superheroes face an adversary that constantly challenges them. The mosquito challenge comes from high reproductive rates, efficient detection of hosts and oviposition, intricate adaptation to aquatic habitats, and intimate damage to humans. How did mosquitoes get to where they are? Evolution has been sculpting mosquitoes for a very long time – at least 90 million years and probably twice that long, compared to our own lineage that is five or ten million years old at most. During those 90 million years mosquitoes as a group have experienced different continental arrangements, different climate regimes, and different hosts – not to mention different pathogens. The current product of that evolutionary process is a moderately sized family of flies that combine an impressive array of general capabilities with supreme specialization for blood feeding. The family Culicidae must be responding to various selective pressures all the time, but we mostly notice when those changes affect humans. Relatively few species are closely tied to human activity, but those that are, like *Anopheles gambiae*and *Aedes aegypti*, probably developed in the last 15,000 years. On a much shorter time scale, the rapid emergence of insecticide resistance is commonly recognized but the examples we have of rapid evolution of biological characteristics is not. Mosquito biologists have shown changes in diapause characteristics of *Wyeomyia smithii* in probably response to climate change and in *Aedes albopictus* in response to its introduction to new geographies, changes that have taken place on the scale of decades rather than millennia. The accelerating pace of mosquito introductions raises the question whether any of these species will become key pests and vectors in their new habitats, much as happened with *Aedes albopictus*. MADs have enough challenges, but mosquitoes as transformers will certainly make our lives interesting.

PL-2 WOTUS and NPDES: Impacts Real and Imagined on Mosquito Control

Carlton Layne

Recent and Emerging Vector-Borne and Zoonotic Disease Threats to the US

1 Chikungunya in the Americas

Steven Higgs, shiggs@k-state.edu, Yan-Jang S. Huang, Dana L. Vanlandingham

Confirmed cases of chikungunya fever were reported for the first time in the Americas during December of 2013. In May of 2014, chikungunya virus was introduced into the United States, with the first autochthonous cases occurring in Florida during July. By the end of 2014 there had been a total of over 2,300 cases in the US. The virus is now likely established in multiple countries throughout the Caribbean and in South and Central Americas with over 1.6M cases

having been reported since the 2013 introduction. The presentation will discuss introduction, establishment and current data.

2 Determining the susceptibility of American *Culex* mosquitoes to Japanese encephalitis virus

Dana Vanlandingham, dlvanlan@vet.ksu.edu, Yank-Jang S. Huang, Susan M. Hettenbach, Julie N. Harbin, Lee W. Cohnstaedt, Alan D.T. Barrett, Stephen Higgs

Japanese encephalitis virus (JEV) is a flavivirus endemic to the Asian Pacific region. Transmission of JEV is constantly maintained among susceptible swine and avian species through the bite of Culex species mosquitoes. Establishment of enzootic transmission cycles in amplification hosts and infected mosquitoes is a critical component required for the dispersal of JEV and other related flaviviruses in the JEV serocomplex. Since its discovery, JEV has been a potential threat for human and veterinary public health in North America due to the presence of large numbers of susceptible swine and avian species. Previous experiments have demonstrated that North American avian species are susceptible to JEV infection; however, the susceptibility of North American Culex species mosquitoes has not been determined. In this study, the susceptibility of Cx. pipiens, Cx. quinquefasciatus, Cx. tarsalis, and Cx. restuans to JEV were evaluated by oral exposure to the virulent genotype-III Taira strain of JEV. Infection and dissemination was confirmed by the quantitative detection of infectious viruses in homogenized tissues. Our results indicate North American Culex species mosquitoes are susceptible to JEV infection.

3 Emerging pathogens associated with the Lone Star Tick, *Amblyomma americanum*

Katherine Sayler, saylerk@ufl.edu, John Lednicky, Anthony Barbet, Jeff Abbott, Michael Dark, Amanda Loftis

The clinical importance and recognition of tick-borne infections has increased dramatically in recent decades. In the United States, tick-borne disease literature has primarily focused on Ixodes spp. as vectors of Lyme disease-causing Borrelia burgdorferi. However, Amblyomma americanum, the lone star tick (LST)—once considered a mere nuisance to people and livestock—has been shown to transmit a number of human and animal pathogens. This aggressive, host-seeking tick is now recognized as a competent vector for both E. chaffeensis and E. ewingii, Rickettsia spp., the Panola Mountain Ehrlichia, Borrelia lonestari, and emerging viruses including the Heartland virus. In the southeastern U.S. this is the tick that most often bites people, underscoring the clinical relevance of this vector. In this study we combined serology, molecular assays, culture isolation and deep sequencing techniques to provide a holistic approach to understanding the pathogens associated with this vector. Using molecular diagnostics we found that 14.6%, 15.6%, and 57.1% of LSTs were qPCR positive for E. chaffeensis, E. ewingii, and Rickettsia spp., respectively. Panola Mountain Ehrlichia and Borrelia lonestari DNA was detected in nearly 2% of tick specimens, respectively. The presence of all five putative pathogens was confirmed using deep sequencing. Furthermore, we were able to isolate an arenavirus, the Tacaribe virus, from a pool of 100 host-seeking lone star ticks. The presence of these organisms in an aggressive tick, often at higher prevalences than previously documented in the literature, highlights their zoonotic potential in the southeastern U.S.

4 Hantavirus pulmonary syndrome and Sin Nombre virus ecology in California

Mark Novakm, Mark.Novak@cdph.ca.gov, Bryan Jackson, Vicki Kramer

Since hantavirus pulmonary syndrome (HPS) was first identified in 1993, more than 70 cases have been identified in California. Most cases of HPS occur as single, sporadic disease events, but an outbreak of 10 HPS cases occurred in 2012 among persons who lodged in a specific type of cabin in Yosemite National Park. Several other HPS cases have been associated with state or federal recreational areas, occurring among visitors and staff of these public land agencies. This presentation will review Sin Nombre virus ecology in California and, using examples from HPS case investigations, summarize activities, areas, and environmental factors that may contribute to elevated disease risk. Collaborative public education and risk mitigation efforts between the California Department of Public Health and public land use agencies will be highlighted.

Adult Control I/ Surveillance

7 Comparison of a novel gas source with CO_2 gas cylinder for adult mosquito surveillance

John Prohaska, jdp@overnia.com, Frank Cornine, Todd Duval

In this paper we investigate the effectiveness of the latest version of the Captivector carbon dioxide source as an adult mosquito lure for CDC light traps. The Captivector system provides mosquito control the ability to deliver carbon dioxide to remote locations where conventional gas systems are not available. In this study the collection efficiencies of the Captivector system and a standard carbon dioxide gas cylinder are compared. We collected mosquito pools from two sites using a random balanced experimental design for the gas sources and collection sites. A total of 36 mosquito pools were collected and analyzed during this study. The data from each gas source and collection location were compared with a two way ANOVA. The results of the statistical analysis indicated no significant difference was observed between the Captivector system and the reference carbon dioxide source. We observed that the variance associated with the collection site locations was greater than the variance from the gas sources. Our results suggest that in this study the Captivector gas source is equivalent to a standard gas cylinder as a mosquito lure with CD light traps.

9 Evaluation of the CDC Autocidal Gravid Ovitrap for the surveillance of La Crosse virus vectors

Brian Byrd, bdbyrd@wcu.edu, Monica Henry, Yanju Li, Sean O'Connell, Charles Sither, Roberto Barrera, Manuel Amador

La Crosse virus (LACv) is the most common cause of pediatric arboviral infection in North America and is endemic in western North Carolina. The virus is primarily vectored by Ae. triseriatus although two invasive species (Ae. albopictus and Ae. japonicus) may be important accessory LACv vectors. Trap based methods for the surveillance of LACv vectors remain inadequate and are currently not suitable for control measures. Here we report the first evaluation of the CDC-AGO within the context of surveillance for LACv vectors. Furthermore, we compared the standard CDC-AGO oviposition attractant, a hay infusion, with a white oak leaf infusion. CDC-AGO traps (n=36) were deployed in a balanced, randomized block design at six peridomestic sites for five weeks (630 trap days per infusion type). Mosquitoes were removed from traps twice weekly, enumerated, and identified by species and sex using microscopic or molecular methods. Overall, the mean yield of LACv vectors was 0.12 mosquitoes per day. The CDC-AGO was highly specific for the three targeted LACv vectors; collectively 94.8% of the trapped mosquitoes were Ae. triseriatus (51.0%), Ae. japonicus (36.1%), and Ae. albopictus (7.7%). There was no detectable difference in the number of mosquitoes collected overall by infusion type. However, contrary to our initial assumptions, our data suggest that the

hay infusion is more effective for trapping Ae. triseriatus than the oak leaf infusion ($p<.05$). There were no detectable species-specific differences in the number of collected mosquitoes by infusion type for Ae. japonicus and Ae. albopictus. Ongoing studies profiling the microbial communities of each infusion and the use of the CDC-AGO as a potential environmental sink are also discussed.

10 The new BG Sentinel 2.0: Comparing old and new Biogents traps and lures

Charles Abadam, cabadam@suffolkva.us, Karen Akaratovic, Jay Kiser

During the summer of 2015, City of Suffolk Mosquito Control conducted studies comparing new and older Biogents surveillance traps and lures in southeastern Virginia. These studies aimed to evaluate the efficiency of Biogents products for field surveillance and determine any species preferences. The first comparison study focused on nightly trapping of the following BG traps: Snap, Sentinel 1.0, Sentinel 2.0, and 2014 Sentinel 2.0 prototype. After preliminary analysis the Snap trap caught significantly less female mosquitoes in terms of both trap totals and Aedes albopictus counts than any of the three Sentinel traps. There were no significant differences between the three Sentinel traps in terms of female totals or female Ae. albopictus counts. These results differ from the 2014 Suffolk studies where significantly fewer mosquitoes were caught in the Sentinel 2.0 prototype when compared to the Sentinel 1.0. The second study compared the new BG Sweetscent lure with the BG lure using both the Sentinel 2.0 and Snap traps nightly. After preliminary analysis there seems to be no significant difference in the female mosquito total counts or female Ae. albopictus counts. In 2014, similar preliminary lure studies conducted with Sentinel 1.0 traps seemed to show a species preference for each lure; Culiseta melanura showed a preference for the BG lure while Ae. albopictus showed a preference for the BG Sweetscent lure.

11 Comparison of carbohydrate sources in yeast-fermentation CO_2 generators for mosquito surveillance

Robert Aldridge, robert.aldridge@ars.usda.gov, Seth C. Britch, Sandra A. Allan, Maia Tsikolia, Lesly Carolina Calix, Kenneth Linthicum

Mosquito surveillance in remote areas with limited access to canisters of CO2 or dry ice will benefit from an effective alternative CO2 source, such as the natural production of CO2 from yeast fermentation of several carbohydrate sources. In this study, we document the differences in mosquito and non-mosquito capture rates from CO2 baited (dry ice or yeast fermentation of carbohydrates) CDC traps over 22 trap nights. Results demonstrated the ability of yeast generated CO2 to effectively attract mosquitoes to a CDC trap, regardless of the carbohydrate source. Total collections of mosquitoes using dry ice were significantly different from and had higher capture rates than yeast generated CO2 sources. There was no significant difference between capture rates of mosquitoes using CO2 generated through yeast fermentation between all three carbohydrate sources. Species comparison and composition will be discussed between carbohydrate sources and dry ice. Volatiles produced by yeast fermentation were also documented by carbohydrate source, and will be discussed regarding synergistic effects with CO2 on mosquito collections

12 Field evaluation of the BG-Counter, a new surveillance tool to remotely measure mosquito densities

Catherine Pruszynski, cpruz@keysmosquito.org, Martin Geier, Michael Weber

The Florida Keys Mosquito Control District (Key West, FL), Biogents AG (Regensburg, Germany) and onVector Technology (Sunnyvale, CA) are working together to develop a remotely-operated trapping device that will provide real-time data on adult host-seeking mosquito populations. The remote automated counting trap has the basic design of the original BG Sentinel trap. Carbon dioxide can be switched on and off remotely to attract mosquitoes. A sensor counts mosquitoes as they enter the trap and can differentiate them from non-mosquito insects, dust, or rain. The trap is self-powered and trap collection data is wirelessly transferred to a district webpage. In March 2015, Biogents provided the first prototype of the trap to FKMCD. Field trials were conducted to test the efficacy and accuracy of the trap collecting in various environments with varying species at different densities. This presentation will review the field experiments used to evaluate the functions of the first trap prototype.

13 A comparison of CDC light trap and Reiter gravid trap data in Vector Index calculations

Broox Boze, bboze@comosquitocontrol.com, Daniel Markowski

Approaches to arbovirus surveillance and adult mosquito control vary significantly by region. The best surveillance programs rely on a thorough understanding of vector biology, disease dynamics, and host-seeking behaviors that affect vertebrate host interactions. These programs also establish localized action thresholds which should take into account spatial and temporal data, so the timing and use of adulticide products is most effective. Throughout Northern Colorado, it is believed that Culex tarsalis and Culex pipiens are the primary vector species for transmitting West Nile virus (WNv). Some municipalities make adult mosquito control decisions based on Vector Index (VI) calculations using the formula provided by Nasci et al. (2003). The host-seeking behavior of these two vector species differs significantly and the perceived abundance of each species in the environment is highly dependent upon trapping methods used. To assess the difference in calculated disease risk, both CDC Light Traps and Reiter Gravid Traps were set at five locations throughout the City of Fort Collins, Colorado and all Culex collected from these locations were tested for WNv using PCR. The VI was then calculated for each trap type at each location, as well as the combination of both types of traps at each location. Preliminary data show that perceived abundance can vary as much as 400 times between trap types. Differences in these abundance data then have profound effects on VI calculations. This may have important public health implications as these data are often used to make decisions about adult mosquito control operations and human risk.

14 Mosquito Bite! A mobile application for tracking mosquito bites

Michael Reiskind, mhreiski@ncsu.edu

We have developed an application for a mobile device that allows the user to simply and automatically report the time and location of a mosquito bite. This piece of information is stored in a database and displayed on a scalable, global map. The advantages of this system are scalability, ease of use, open access to data, and accurate time and place measurements. Disadvantages are a lack of species specific information, gaining widespread use, and the risk of abuse. Solutions to the disadvantages include adding site/time specific pest information, intensive marketing campaigns, and limitations on reporting time frames.

Biology and Behavior I

15 DDT and land use, not global warming, drives broad scale changes in mosquito populations over the past century

Ilia Rochlin, ilia.rochlin@suffolkcountyny.gov, Dominick Ninivaggi, Ary Faraji, Christopher Barker, A. Marm Kilpatrick

Recent shifts in geographic distributions of many insects have coincided with increased temperatures, raising the possibility that climate change may cause mosquito vectors of diseases such as dengue or malaria to expand their range poleward or upward in elevation. Other environmental factors, including land and pesticide use, have also changed over the past century but have been largely ignored in analyses of insect population trends. We examined associations between temperature, DDT (dichlorodiphenyltrichloroethane) use, urbanization, and mosquito abundance across eight decades on the east and west coasts of North America. We show that mosquito communities fluctuated enormously with species richness and abundance varying over an order of magnitude. Changes in mosquito populations were mostly uncorrelated with annual temperature and instead, urbanization and DDT use from the1940s to the1960s followed by decaying persistence in the environment thereafter, were the main drivers of long term dynamics of mosquitoes. These results demonstrate the lasting impacts of anthropogenic chemical use on ecosystems. They also highlight the potential for false attributions to climate change in studies of insect populations and distributions.

16 The North American Mosquito Project (NAMP) 2015 update: *Aedes vexans* population structure

Lee Cohnstaedt, Lee.Cohnstaedt@ars.usda.gov, Phillip Schumm

In 2011 and 2012, the North American Mosquito Project used professional mosquito control agencies and citizen scientists to collect 1,101 distinct collections of mosquitoes and the sampled distributions covered the entire known distribution of Culex tarsalis and Aedes vexans in the continental United States. With this “snap-shot” of genetic material, population- and phylo-genetic analysis identified the geographic origins of the species. The Culex tarsalis populations were analyzed in 2014. In 2015, the Aedes vexans populations were used for genetic analysis to determine discrete populations and phylogenetic relationships.

17 The genus *Aedes* nearly disappears but is rediscovered in plain view

Richard Wilkerson, wilkersonr@si.edu, Yvonne-Marie Linton, Dina Fonseca, Ted Schultz, Dana Price, Daniel Strickman

The tribe Aedini (Family Culicidae) contains approximately one-third of all known species of mosquitoes, including vectors of deadly or debilitating disease agents. This tribe contains the genus Aedes, which is one of the three most familiar genera of mosquitoes. During the past decade, Aedini has been the focus of a series of extensive morphology-based phylogenetic studies published by Reinert, Harbach, and Kitching (RH&K). Those authors created 74 new, elevated or resurrected genera from what had been the single genus Aedes, almost tripling the number of genera in the entire family Culicidae. The proposed classification is based on subjective assessments of the “number and nature of the characters that support the branches” subtending particular monophyletic groups in the results of cladistic analyses of a large set of morphological characters of representative species. We reanalyzed their data and found that their phylogeny was largely weakly supported and their taxonomic rankings failed useful taxon-naming criteria. Consequently, we propose to: 1) restore an aedine classification system that is useful for the operational community; 2) enhance the ability of taxonomists to accurately place new aedine species into genera; 3) maintain progress toward a natural aedine classification; and 4) correct the current classification system. We do not challenge the phylogenetic hypotheses in the above-

mentioned series of studies. However, we reduce the ranks of the genera and subgenera of RH&K to subgenera or informal species groups, respectively, to preserve the stability of genus Aedes, and as a result enhance communication and the exchange of information.

18 Temperature dependent development rates of container-inhabiting mosquitoes

Kristen Healy, khealy@lsu.edu, Emily Boothe, Dina Fonseca

Temperature is an important factor that contributes to the development rate differences in mosquitoes. While many other factors contribute to development (such as diet and crowding), temperature-dependent development rates can be used to make predictions regarding species development and abundance. Our lab is currently evaluating the temperature-dependent development rates of container-inhabiting mosquitoes, including a comparison of Aedes albopictus populations from Louisiana and New Jersey. Data from these studies can be used to further enhance degree-day models that were initially developed as part of the Area-wide management of Aedes albopictus project that was conducted in New Jersey.

19 The Siren's Song: Exploitation of female flight tones to passively capture male *Aedes aegypti* mosquitoes

Brian Johnson, brian.johnson@jcu.edu.au, Scott Ritchie

The need to capture male mosquitoes has intensified recently as a result of a number of male-based sterile insect technique (SIT) and population modification programs focused on Aedes aegypti having initiated field releases. Here, we report the results of the successful exploitation of the attraction of male Ae. aegypti to female flight tones to enhance male collections in passive Gravid Aedes Traps (GAT). Prior to field studies, male attraction to female flight tones of 484 and 560 Hz and to a male flight tone of 715 Hz was assessed in a series of controlled release-recapture and semi-field trials. These trials determined that a pure tone of 484 Hz was significantly ($F_{3,24}=5.04$, $P=0.01$) more attractive to free-flying males than the other flight tones and enabled their collection in sound-baited GATs (ca. 95% capture after 2 h; 484 Hz at 65 dB). In contrast, gravid females were unresponsive to male or female flight tones and were equally distributed among sound-baited and control GATs. Importantly, under normal field conditions sound-baited GATs (484 Hz, 70 dB) captured significantly ($t_8=4.01$, $P=0.01$) more male Ae. aegypti per 24 h trap interval (1.3±0.37) than controls (0.2±0.13). Overall, sound-bated GATs captured approximately twice as many Ae. aegypti (male and female) (3.0±0.68 per interval, 30 total) than controls (1.5±0.56 per interval, 15 total). These results reveal that sound-baited GATs are an effective surveillance tool that would allow male-based SIT and population modification programs to effectively monitor their target populations. Furthermore, the practicality of sound-baited GATs may extend to other day active, container-utilizing members of the Stegomyia subgenus, such as Aedes albopictus and Aedes polynesiensis.

20 Florida Keys Mosquito Control District and the USDA collaborating to bring an understanding of invasive mosquito species to community schools

Beth Ranson

209 Field evaluation of three traps used for collection of host-seeking *Culex quinquefasciatus* in Louisiana

Emily Boothe, emilyb@lsu.edu, Kristen Healy

In order to effectively quantify *Culex quinquefasciatus* population decline after a spray event, we must be able to capture host-seeking females. Currently, our most effective trap for collecting

females is a gravid trap. The purpose of this study was to evaluate which traps would be useful in collecting host-seeking female *Cx. quinquefasciatus.* This study was conducted from June 8th to 12th 2015 at six sites in three Louisiana parishes. Two carbon dioxide-baited traps, Centers for Disease Control (CDC) miniature light trap and BioGents Sentinel (BGS) Mosquito Trap, were compared with gravid trap collections. Four treatments were used for the CDC light trap: incandescent, red, ultraviolet and no light. Three treatments were used for the BGS traps: BG Lure, Octenol and no lure. Captured female *Cx. quinquefasciatus* were counted and scored using the Sella score of bloodmeal digestion (1-7) and identified as unfed and host-seeking (1), bloodfed (2-6) or gravid (7). Results indicate gravid traps and CDC light traps with no light most effectively sample host-seeking females.

Student Competition I

27 Chikungunya and dengue mosquito resting and sugar-feeding behavior in urban areas of Ecuador

Diana Naranjo, dianitapaola@hotmail.com, Whitney Qualls, John Beier, Eduardo Gómez, Stephanie Pinela, Virginia Pinela

Abstract In Durán, Ecuador, newly urbanized areas provide distinct housing qualities (HQs) that may impact Aedes aegypti behavior. We defined HQ by the type of construction material of the house. Moreover, there are numerous abandoned vegetated lots (VL) providing mosquito-vectors with resting and sugar-feeding habitats. We aim to estimate proportions of Ae. aegypti female mosquitoes that sugar-feed in VL accounting for HQ. We selected nine 50x50 VL and recorded the construction material of the homes that were within 30 m distance from the VL. A barrier application of a non-attractant color-dyed 30% sucrose solution was made to the VL. The dye allowed tracking of mosquitoes that were resting or sugar-feeding in the VL and then entered the houses. Post 24h application, indoor aspirations were conducted. We gave a HQ score of 0 if the construction materials were cane, wood, and dirt or a score of 1 if it was cement; scores were added. Counts of total female mosquitoes stained and non-stained were recorded. The mean number of stained females adenines found was 1.76 (SE± 0.39; CL 0.91–2.62) from a total mean of 3.53 (SE ±0.39; CL:1.94-5.12). Correlations between HQ and proportion of stained females were found (r2=0.56; p=0.04). HQ was not associated with total proportion of females. Evaluation suggests that sugar-fed female Ae. aegypti may prefer to switch from VL as resting habitats to homes with a better HQ. More detailed studies are needed to predict Ae. aegypti abundance in reference to construction materials and sugar availability in urban areas.

66 Adding an IGR to Mosquito Barrier Treatments to Increase Their Residual Effectiveness in Suburban Backyards

Glen Skiles, glenn.skiles@uky.edu, Kyndall Dye, Nicola Gallagher, Grayson Brown

Aiming to increase residual effectiveness of mosquito barrier treatments, the pupal IGR Archer® was combined with Demand® and applied to the perimeter of 30 suburban backyards. Mosquito populations were then monitored weekly using 5 different methods and homeowners surveyed three times. Mosquito populations were reduced by approximately 75% compared to controls and remained suppressed throughout the study period. Surveys revealed a high level of homeowner satisfaction, although a placebo effect was evident in their responses.

22 Modeling optimum use of attractive toxic sugar bait stations for effective malaria vector control in Africa

Lin Zhu, l.zhu3@med.miami.edu, John Marshall, Whitney Qualls, Yosef Schlein, John Beier

Insecticide resistance and the increased outdoor biting behavior of malaria vectors reduce the efficiency of indoor vector control methods. Attractive toxic sugar baits (ATSBs), a method targeting the sugar feeding behaviors of vectors both indoors and outdoors, is a promising supplement to indoor tools. A hypothetical village consisting of houses, humans, and essential resource requirements of Anopheles gambiae (sugar sources, outdoor resting sites and larval habitats) was simulated in a spatial individual-based model to determine the optimum number and configuration of ATSB stations needed for malaria control. Compared to the outcomes not altered by the ATSB treatment in the control scenario, the optimum ATSB treatment reduced female abundance by 98.2%, reduced human biting rate (HBR) by 99.5%, and reduced entomological inoculation rate (EIR) by 99.9%. N x n grid design, stations at sugar sources, resting sites, larval habitats, and random locations worked better in reducing vector population and HBRs than other configurations ($P < 0.0001$). However, there was no significant difference of EIR reduction between all ATSB configurations ($P > 0.05$). ATSB treatment reduced An. gambiae population substantially and reduced EIR to near zero regardless of environmental resource availability. Dispersive configurations worked better in reducing vector population, and stations at or around houses worked better in preventing biting and parasite transmission. Optimum numbers of bait stations should be adjusted according to seasonality when resource availability changes.

23 Sublethal effects of Temprid® on bed bug (*Cimex lectularius)* behaviors and implications for control

Sydney Crawley, secraw2@uky.edu, Michael Potter, Kenneth Haynes

Exposure to a sublethal dosage of insecticides can often result in changes in the insect's behavior. These behavioral changes observed in individuals may have consequences at the population level, and thus could impact success or failure of pest management. In this study, we investigated the sublethal effects of Temprid®, a product that combines a neonicotinoid and a pyrethroid insecticide, on various bed bug (Cimex lectularius) behaviors. Bed bugs are an urban pest with public health relevance, as infestations result in substantial effects on human health and well-being. We found that short-term exposure to a residual deposit of this insecticide resulted in a reduction in the volume of blood taken per blood meal, and reduced efficiency of host finding. Additionally, the Temprid® reduced spontaneous movements of bed bugs that are under circadian control. Fecundity of insecticide-exposed bed bugs was also reduced. However, we found no difference in the ability of treated bugs to respond to bed bug aggregation pheromone; exposed bugs located a marked harborage as efficiently as unexposed bugs. Also, treated and untreated bed bugs did not avoid insecticide deposits. These results were consistent among three populations of bed bugs with varying inherent levels of insecticide susceptibility. These data suggest that the behavior and fecundity of bed bugs can be adversely affected by exposure to an insecticide. Implications of sublethal effects of insecticides on pest management of bed bugs and other blood-feeding arthropods will be discussed.

Education/ Public Relations/ General

44 Mastering the mosquito control message and media interview

Jill Oviatt, joviatt@cvmvcd.org

The quality of your spokespeople and their ability to communicate important messages effectively and manage risks during media interviews can make or break the reputation of a Mosquito and Vector Control District and dictate whether the public understands and acts on specific threats to public health. Knowing how to craft the right message for a media interview,

deliver that message repeatedly with the right tone and manner, and knowing how to answer tough questions without becoming defensive, while maintaining control of the interview and bridging back seamlessly to your message are all skills spokespeople need to be effective in communicating to the public during interviews. This paper outlines a step-by-step process for managing media interviews, including how to develop and use messages that are newsworthy; how to control and manage interview flow; and how to handle difficult questions with confidence. Examples of messaging crafted at mosquito and vector control districts and the interview clips that resulted will be used to illustrate the method.

45 Making the mosquito the hero – The shaping of a public relations campaign

Kelly Middleton, kmiddleton@glacvcd.org, Jason Farned, Levy Sun

Two Los Angeles County, California, vector control districts collaborated with the University of Kentucky and MosquitoMate on the release of the novel Wolbachia-infected male Aedes albopictus (Asian tiger mosquito) or 'ZAP' mosquitoes to evaluate this technology for the control of a rapidly spreading infestation of Aedes albopictus in parts of Los Angeles County. Creating an effective outreach strategy required that these districts introduce this exciting new program, convey the need, the strategy, the safety, and the goals of this approach in an easy-to-understand and palatable manner. This campaign targeted an extremely diverse demographic from community leaders to non-English speaking residents. Developing the Sterile Male Tiger Mosquito Pilot Program campaign required careful attention to specific message terminology. The campaign was implemented in three key phases to guarantee adequate notice and ease potential concerns over the use of a novel strategy to control mosquitoes in densely populated Los Angeles County. Careful priming and message management yielded a successful campaign launch and widespread public support.

46 Clicking to the masses

Adrian Salinas, asalinas@cmcd.com

Every mosquito control public information department can be its own TV station, magazine, and newspaper with a website, Facebook and Twitter. Are you preparing your surveillance equipment for the season? Did an inspector find larvae? Did you collect 12,000 adults in a surveillance trap? These are perfect activities to post. Shoot some video add content and post it! By posting current activities in a timely manner, it illustrates the scope and complexity of an integrated mosquito management program.

210 Pennies per person: Digital advertising closing the gap between people and vector control

Levy Sun, lsun@glacvcd.org

There are numerous studies on the effectiveness of public health agency marketing and educational outreach strategies. However, there is a lack of knowledge about the effectiveness of newer, digital tools to communicate to vector control agencies' target audiences. One of these tools is targeted online advertising (TOA) utilizing audiences' Internet search queries and their geographic locations. Extant literature reveals that there are significant benefits to using TOA in general marketing outreach. Agencies with limited budgets can engage multiple audiences over a large geographic area. Additional benefits include displaying a vector control's key messages in defined parameters as narrow as zip codes and demographic characteristics. This allows vector control to target "hotspots" within the agency's jurisdiction. From 2014 to 2015, Greater Los Angeles County Vector Control District (GLACVCD) implemented TOA to determine its

efficacy in public relations and marketing strategy. Analysis of TOA results revealed many benefits for vector control agencies. This digital tactic provided real-time results, which allows vector control communicators to quickly adjust public relations and marketing strategies. The cost-benefit analysis showed GLACVCD's TOA tactic reached its target audiences at the average cost of a $0.013 USD per person for a total of more than half a million impressions. Additionally, TOA, in combination with analytic tools, helped measure the efficacy of other public relations tactics. Analyzed data also provided suggestions for future research.

211 REPEL: (Reach out, Educate, Provide, Everyone, Local)

Cindy Mulla, cindymulla@comcast.net

Strategy building from your base camp (mosquito control district) to reach the summit (the citizens) by educating and providing your local expedition (community) with accurate information. This method can be continuously acquired by a multitude of unique and fascinating routes. Thus, creating the comfort level for the individual through REPEL (Reach out, Educate, Provide Everyone Local). It's even possible to reach the national and international level through this positive and trusting established relationship. Within our district, REPEL displayed success and we would like to share this method with the industry.

Recent and Emerging Vector- Borne and Zoonotic Disease Threats to the US Continued

5 Factors affecting the ability of American mosquitoes to transmit Rift Valley fever virus

Mike Turell, michael.j.turell.civ@mail.mil

As indicated by West Nile virus and the recent introduction of chikungunya virus into the Americas, exotic viruses have the potential to spread to previous uninfected areas and cause significant disease and economic disruption. Of particular concern is Rift Valley fever virus (RVFV), a bunyavirus found throughout Africa that causes significant disease in both domestic animals and humans. Various studies have indicated that numerous North American mosquito species are competent vectors, should RVFV make it to the Americas. In this presentation, I will cover various factors that affect the ability of mosquitoes to transmit RVFV. These range from environmental temperature to effects of RVFV infection on the behavior and physiology of vertebrate hosts. In addition, infection of the arthropod vector with RVFV can affect its vectorial capacity, and the presence of other organisms, such as microfilariae in a vertebrate host with a RVFV viremia, can dramatically affect the ability of mosquitoes feeding on that host to transmit RVFV. A better understanding of the factors affecting the ability of mosquitoes to become infected with and to transmit RVFV is critical to developing an effective response plan.

6 Rift Valley fever in the US: Commerce, networks, climate and susceptible vector and host populations

Seth Britch, seth.britch@ars.usda.gov, Kenneth J. Linthicum, Assaf Anyamba, Andrew J. Monaghan

Rift Valley fever (RVF) is a mosquito-borne hemorrhagic viral disease with substantial negative impacts on public and animal health in its endemic range of sub-Saharan Africa. Rift Valley fever virus (RVFV) could enter the United States and lead to widespread morbidity and mortality in humans, domestic livestock, and wild ungulates, with high economic loss. Some US mosquito

species have been identified as potentially competent vectors for RVFV, some US regions have favorable climate for mosquito development and possibly RVFV transmission in months with historically intense RVFV activity in the endemic range, and connectivity exists between RVFV endemic regions and the US. To reduce the likelihood of RVFV introduction to the US, and to understand the capacity of the virus to spread geographically if introduced, we investigated techniques to identify key US locations vulnerable to RVFV incursion based on historical population dynamics of potentially competent US mosquito vectors and their relationship to historical meteorological and satellite environmental data, based on patterns of US climate during RVF outbreaks in endemic regions, and based on connectivity to RVF endemic regions. We derived indices of environmental patterns that historically precede unusually high populations of potential US mosquito vectors of RVFV with lead times of weeks to months. Combined with monitoring of RVF activity in endemic regions and nodes of connectivity, environmental monitoring in the US in high risk locations could be used to guide targeted surveillance and control of potential US RVFV mosquito vectors.

143 Factors that influence the transmission of West Nile virus in Florida

Jonathan Day, jfda@ufl.edu

West Nile virus (WNV) has become well established throughout much of North, Central, and South America where it has been responsible for widespread and extensive epidemics in human populations and epizootics in domestic animals and wildlife. In Florida, WNV was first detected in 2001. Despite a history of epidemics caused by St. Louis encephalitis virus in Florida, extensive WN epidemics have not yet been reported in the state. The reasons why WNV has not caused epidemics in Florida remain unclear but likely center on the interaction of vector mosquitoes, avian amplification hosts, and the environmental conditions that impact the vector and amplification host populations. The primary vector of WNV in Florida is Culex nigripalpus Theobald. Rainfall, drought, and temperature are the primary factors that regulate annual populations of this species. Evidence suggests that a combination of environmental factors influencing Cx. nigripalpus oviposition, blood feeding behavior, and vector competence have limited WNV transmission in Florida to relatively small focal outbreaks and kept the state free of a major epidemic.

144 Zika virus: A new vector-borne scourge in the Americas

Harry Savage, hms1@cdc.gov

Zika virus is a flavivirus related to West Nile, dengue, and yellow fever viruses. From its first isolation in Uganda in 1947 until 2007, only 14 cases of human Zika virus infection were reported and no transmission of the virus had been noted outside of Africa and Asia. In 2007, a Zika virus outbreak occurred on Yap island in Micronesia, which affected approximately 73% of the island's population. Subsequently, outbreaks have occurred in several Pacific islands, including French Polynesia, New Caledonia, French Polynesia, Easter Island, Philippines and Vanuatu. The first Zika virus outbreak in the Americas was noted in Brazil in May 2015. The virus has subsequently spread in that country causing tens of thousands of cases, but as of September 2015 transmission has not yet been confirmed elsewhere. Aedes aegypti efficiently transmits Zika virus, and as such, the virus is likely to spread throughout the region in a similar manner to chikungunya. Clinically the virus presents as a mild dengue-like syndrome with rash, fever, arthritis or arthralgia, and headache as common features; however, unlike dengue, Zika rarely results in hospitalization. Diagnosis by serology is complicated by the cross-reactivity of dengue and Zika antibodies, but definitive diagnosis can be made by nucleic acid detection tests.

Student Competition II

24 **Quantitative analysis of vector behavior following subacute expose to prallethrin, an active ingredient in Duet®**

Kyndall Dye, kyndall.dye@uky.edu, Grayson Brown, Kenneth Haynes, Douglas Johnson

A laboratory spray system was constructed simulating a ULV application of technical prallethrin to adult female mosquitoes. Treated mosquitoes significantly increased turning rate, distance, velocity, and number of flight events, a response that would increase exposure to a ULV application. However, this behavioral response was not seen in non-contact trials because prallethrin has low volatility. These results suggest that ULV adulticides with a behavioral modifying chemical more volatile than prallethrin would be more efficacious.

25 **Blood-feeding behavior of Puerto Rican Aedes aegypti exposed to pyrethroid-treated fabric**

Natasha Agramonte, nme@ufl.edu

Pyrethroid resistance, a vector control problem, has plagued Puerto Rican Aedes aegypti for decades. This study compared the blood-feeding behavior in pyrethroid-resistant and susceptible strains of Ae. aegypti. Uniform fabrics treated with four pyrethroids were assayed on volunteers' forearms for 15 min periods. Dose-response curves were generated for each chemical pair and resistance ratios were calculated from the LD50 values. Variable ratios were observed based on the pyrethroid type.

26 **Chemical compounds identified in domestic dog odors**

Chris Holderman, chrish2@ufl.edu, Philip Kaufman, Matthew Booth, Ulrich Bernier

Mosquitoes transmit Dirofilaria immitis to 750,000 dogs annually, yet little is known of the chemical cues used by mosquitoes for host location. With IACUC approval, we used sorbent tubes and a vacuum pump to collect volatile organic compounds (VOCs) emitted from the fur of 10 dogs. The tubes were then analyzed using a thermal desorption/gas chromatograph/mass spectrometer. These VOCs will be evaluated for their ability to attract Aedes albopictus in a laboratory olfactometer.

21 **Terpenoids are capable of enhancing synthetic insecticides against *Aedes aegypti***

Edmund Norris, ejnorris@iastate.edu, Lyric Bartholomay, Joel Coats

The burden of mosquito-borne disease to public health throughout the world cannot be underestimated. Every year, approximately 700,000 people die from complications associated with etiologic disease agents transmitted by mosquitoes. With insecticide-resistant mosquito populations becoming an ever growing concern, the need for new insecticidal formulations is more important than ever. We screened synthetic pyrethroids (permethrin, deltamethrin, B-cyfluthrin, and natural pyrethrins) and other synthetic insecticides with various essential oils in order to enhance the efficacy of these insecticides. From this data, we hypothesize that plant essential oil components are capable of enhancing the effect of various synthetic insecticides by interfering with detoxification enzymes. In order to better understand this phenomenon, we have begun to isolate fractions from essential oils that are the most promising enhancers of insecticide toxicity. This should identify various plant-derived compounds which could be important in the discovery of novel synergistic compounds or additives to future insecticides. Moreover, our work will lead to the screening of other plant essential oil and synthetic insecticide mixtures against

insecticide-resistant strains of mosquitoes. This research may prove crucial to the future of vector control.

28 Ecology of La Crosse Virus (LACv) vectors along forest-to-field ecotones in western North Carolina

Marcelo Schwarz, m_schwar@uncg.edu, Gideon Wasserberg, Brian Byrd

La Crosse Encephalitis (LACE) is a pediatric disease with a recent emergence in the number of reported cases in Appalachia. This increase may be due to improved reporting and/or a greater exposure to La Crosse virus (LACv) vectors. The LACv is historically transmitted by the sylvan eastern tree-hole mosquito (Aedes triseriatus). However, recently invasive species (Ae. albopictus and Ae. japonicus), likely secondary peri-domestic vectors known to co-occur with Ae. triseriatus, complicate the understanding of LACv ecology. Study goal: Determine the effect of landscape structure (i.e., forest-to-field ecotones) and artificial container introduction (i.e., tires) on the distribution and abundance of the LACv in western NC. Hypotheses: 1. Canopy-associated environmental variables determine LACv vectors' distribution and clustering along these ecotones; 2. Tire introduction increases local (habitat-specific) and overall (across ecotone) abundance of LACv vectors. Methods: We ran 2 parallel transects per site (6 sites total), each 200-meters in length, 15 ovitraps per transect; we also deployed traps for gravid (BG Sentinels and Landing-Biting) and resting (Nasci aspirator) mosquitoes. We incorporated 9 tires in each experimental plot: 2 sites received treatment in the field, 2 sites in the forest, and 2 sites served as control. Preliminary results suggest habitat preferences with Ae. albopictus more abundant in the field habitats, and Ae. japonicus as well as Ae. triseriatus more common in the forest and edge habitats. The artificial container introduction appeared to increase the abundance of all species, particularly in their "preferred" habitats; however, it did not result in altered oviposition patterns along the ecotone.

29 Development of a novel molecular method for La Crosse virus detection from Aedes mosquito vectors in Knox County, TN

Cassandra Urquhart, curquhar@vols.utk.edu, Doris D'Souza, Amy Lambert, Rebecca Trout-Fryxell

La Crosse virus (LACv) is transmitted via the bite of the primary vector, Aedes triseriatus, and secondary vectors Ae. albopictus and Ae. japonicus. The virus is the leading cause of arboviral encephalitis in children. The most severe cases, which can include permanent brain damage or death, most often occur in individuals under 15 years of age. No vaccines or antivirals exist, making accurate surveillance and prevention methods crucial for protecting human health. The standard molecular method for LACv screening is reverse transcriptase polymerase chain reaction (RT-PCR), an effective yet somewhat time consuming and expensive procedure. Additionally, mosquito abatement districts and county health departments often lack resources for performing these molecular tests. We hypothesize that an economical, isothermal (eliminating the need for PCR machines), sensitive, specific, and faster molecular method for the detection of LACv from vectors and reservoirs can be developed. Consequently, positive controls obtained from the CDC were tested using the known/standard RT-PCR protocol and compared with a newly designed procedure. Varying concentrations of reagents, including primers, buffer, and enzymes, in the novel reaction mix were tested against RT-PCR for efficacy and sensitivity. Once virus was routinely detected from positive controls the novel method was compared to RT-PCR for LACv detection from vectors collected from sites around Knox County, Tennessee. The development of a novel LACv detection method provides a rapid and cost-effective monitoring tool for mosquito

abatement districts and county health departments, which may lack the resources for expensive RT-PCR equipment.

Adult Control II/ Repellents/Chemical

30 Evaluation of repellent-treated U.S. military combat uniforms

Ulrich Bernier, uli.bernier@ars.usda.gov, Natasha Agramonte, Melynda Perry, Amy 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 U.S. 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 their combat uniforms in 2009. Over the past few years, an increasing proportion of combat uniforms are 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 and etofenprox in 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.

31 OFF! Clip-on repellent device with metofluthrin tested on Aedes aegypti (Diptera: Culicidae) for mortality at different time intervals and distances

Christopher Bibbs, cbibbsamcd@bellsouth.net, Rui-De Xue

The OFF! Clip-on mosquito repellent device was tested in outdoor conditions against Aedes aegypti (L.). A single treatment device was suspended 1 m from ground level. Cages of Ae. aegypti were suspended 1 m above ground level in batches of four cages each at three locations 0.3 m from treatment epicenter, 0.6 m and 0.9 m. Exposure times were sectioned into 5-minute, 15-minute, 30-minute, and 60-minute test groups. Initial knockdown and mortality after 24 hours was recorded. The devices had effective knockdown and mortality, but were not sustained at distances greater than 0.3 m from the device. The OFF! Clip-on with 31.2% metofluthrin successfully relieves biting pressure as an insecticidal agent.

32 Relative efficacy of novel spatial repellents for mosquito control

Joel Coats, jcoats@iastate.edu, Edmund Norris, James Klimavicz, Aaron Gross

Spatial repellents hold promise for suppressing adult mosquito movement into premises, including houses, barns, yards and parks. Sesquiterpenoids with relatively low vapor pressures can provide repellency as they emanate off a surface. A laboratory spatial repellency chamber serves as a bioassay for comparison of plant essential oils and individual sesquiterpenoids isolated and purified from those oils. Comparisons are made with two monoterpenoid commercial standards citronella and p-menthane-3,8-diol, as well as the pyrethroid allethrin. Effective spatial repellency lasts much longer than the two comparison terpenoids. The potential for spatial repellents in vector control programs is discussed.

33 Research study effective for mosquito repellent products in Thailand, with Aedes aegypti mosquito resistant strain and susceptible strain of pyrethroid chemical groups

Benjawan Tuetun, benjawanck@hotmail.com, Atthapong Chumkiew, Anuluck Junkum, Daruna Jumpakaew, Benjawan Pittasawat

Research study repellent product on the market in Thailand of the 30 products contained in the main component is DEET (N, N-diethyl-m-toluamide) and chemical synthesis standard DEET in different concentrations of Ae. aegypti susceptible and resistant strains to pyrethroid chemical group, under laboratory. The protection time of mosquito repellent spray products which 16 products, the concentrations of DEET 12-95% for protection time 1.5(1-1.5)-6.5(5.5-6.5) hours which protection time is shorter mosquito 0.5(0.5-1)-6 (5.5-6.5) h. to Ae. aegypti susceptible and resistant strains to pyrethroid chemical group, respectively, For mosquito repellent lotion products which 14 products the concentrations of DEET 7.5-. 50% for protection time 1(0.5-1)-4.5(4-4.5) h. which the protection time is shorter 0.5(0.5-1)-3(3-4) h. to Ae. aegypti susceptible and resistant strains to pyrethroid chemical group, respectively. The solution concentrations of DEET 10-95% for protection time 2(2-2)-7(6.5-8) h. which the protection time is shorter 1.5(1-1.5)-6(6-7) h. Ae. aegypti susceptible and resistant strains to pyrethroid chemical group, respectively. The protection time of mosquito repellent products on the market by a mosquito repellent DEET is the main ingredient in 30 product and solution standards, insect repellent DEET in concentrations. There mechanism of resistance to insect repellent DEET in a group of Ae. aegypti strains resistant to pyrethroid chemicals group. Protection time of mosquito repellent products or standard solutions prepared and tested in time to protect mosquito bites which protection time of decline when compared with species of Ae. aegypti susceptible to pyrethroid chemical groups.

34 Next generation of controlled release microdevices for field and personal protection against mosquitoes

Noel Elman, noel.elman@gmail.com, Ulrich Bernier, Melynda Perry, Craig Scoops, Daniel Kline, Pablo Gurman

Controlled Release Devices (CRDs) for Field and Personal Protection were developed for for optimized spatial coverage of insecticides, maximizing protection and limiting toxicity. CRDs for Field Protection (CRDs-FP) were designed for semi-outdoor environments, e.g. tents, with areas of about 25 m2. CRDs for Personal Protection (CRDs-PP) were designed for fabrics integration, e.g. Army uniforms, for over 2 week protection. CRDs rely on the use of an exothermic reaction to accelerate dispersion and a permeable set of membranes to perform controlled release of chemicals in air. CRDs were prototyped using state-of-the-art SLA 3D printing and subsequently manufactured using cost-effective micro-injection molding technology, defining polymeric micro-structures. CRDs were also implemented with biodegradable polymers. CRDs-FP and CRDs-PP were tested against A. Aegypti and currently being tested against Anopheles. A number of insecticides and repellents were integrated with CRDs including: Transfluthrin, Metofluthrin, and Niaouli. Entomological experiments included testing CRDs-FP in a standard military tent with approximately 320 mosquitoes. Preliminary results showed that the use of CRDs-FP with metofluthrin resulted in a mortality of approximately 98 % in a 2 hour period. Additionally, CRDs-PP were tested in small cage experiments, showed that CRDs-PP with transfluthrin resulted in a reduction in number bites of approximately 90 % for a three week period. A new set of experiments have been conducted at NAMRU-6 to test CRDs against vector species in the South American Region, including Anopheles darlingi. CRDs represent the next generation of devices that leverage the use of advanced engineering to optimize vector control.

35 **Exploiting the K&D in vitro bioassay system for evaluating new mosquito control products**

John Smith, docmx8@gmail.com

This presentation will discuss manipulations made to the K&D bioassay system for evaluating repellents and feeding deterrents against Aedes aegypti. Results will be presented on a variety of experimental DEET-alternative products including a new feeding deterrent mixture.

36 **Metofluthrin emanators reduce *Aedes aegypti* survival and biting intensity: results of field trails in Cairns, Australia**

Jonathan Darbro, Jonathan.Darbro@qimr.edu.au, Ordwell Muzari, Arthur Giblin, Scott Ritchie, Greg Devine

Spatial repellents such as metofluthrin have potential as an alternative method of Aedes aegypti control compared to indoor residual spraying. Studies in simulated indoor environments treated with a metofluthrin-impregnated plastic emanator show that metofluthrin causes knockdown of Ae. aegypti and reduced biting. Here, we extended these studies to actual rooms in three houses in Cairns, Queensland. Each treated room had one plastic emanator and two 30cm3 cages with female Ae. aegypti at 1m and 3m from the emanator. Knockdown was measured via visual count over 2 hours, and biting intensity was measured by the number of mosquitoes landing and probing on a gloved hand within 2 minutes. Results showed high variability (0 – 80%) in knockdown depending on room. Reduction in biting intensity was more uniform: a biting reduction of 70-80% was observed after 10 minutes and 90-100% after 30 minutes (depending on room). Mosquitoes 3m away from an emanator in a highly open space (i.e. porch or veranda) tended to show no differences from untreated controls. Similar trials in Brisbane, Australia demonstrate similar results for salt marsh and freshwater mosquitoes such as Ochlerotatus vigilax and Culex annulirostris. These results suggest that hanging emanators in rooms where people and Ae. aegypti are likely to interact could be an efficacious method of preventing biting and thus could improve efficiency of dengue control programs in northern Australia.

37 **Comparisons of the efficacy of Duet™ and Kontrol 30-30® in Lee County, Florida**

Rachel Morreale, morreale@lcmcd.org, T. Wayne Gale, Jonathan Hornby

Duet™ was compared to Kontrol 30-30® in treatment applications in Lehigh Acres and in Fort Myers, FL. The differing locations were selected to test the products in two of the different habitat types found in Lee County. The developed and densely populated Fort Myers locations contrasted the urban environment with the undeveloped and sporadically populated Lehigh Acres locations. Duet™ was applied at a flow rate of 0.75 oz/ac with 0.0044 lb Prallethrin/ac and 0.0022 lb Sumethrin/ac. Kontrol® 30-30 was applied at a flow rate of 0.33 oz/ac with 0.006 lb Permethrin/ac. The order of products for initial applications were randomly selected for each area and switched for a subsequent treatment. In order to rule out population declines due to a trapping out effect, traps were employed at varying intervals. BG-Sentinel traps were used to catch mosquitoes in selected areas for three nights before treatment application and for three nights following the treatment. Mosquito samples were identified to species. Product efficacy was determined by comparing mosquitoes caught before and after treatment events.

212 **Novel compounds to control susceptible and resistant population of An. Gambiae**

Aaron Gross, adgross@epi.ufl.edu, Fan Tong, Rafique Islam, Baonan Sun, Paul Carlier, Jeffrey Bloomquist

Anopheles gambiae mosquitoes are a significant vector of malaria in Sub-Saharan Africa, and the use of chemical insecticides is a common method to help control malaria by killing the mosquito vector. However, mosquito resistance to insecticides has resulted in a clear and urgent need to develop new effective chemical insecticides. Potassium channels provide an alternative target for mosquito control. Potassium channels function as a regulator of excitation in the insect's central and peripheral nervous system, and at the insect's neuromuscular junction. Screening of several hundred novel potassium channel blockers was performed *in* vitro using whole-cell patch clamp. Additionally, *In vivo* toxicity of novel potassium channel blockers was also performed against adult *An. gambiae* mosquitoes including a susceptible strain (G3), and an *An. gambiae* strain with enhanced P450 activity (Akron). The topical toxicity of five potential potassium channel blockers ranged from 68 ng/mg to 356 ng/mg. Four out of the five potential potassium channel blockers did not display cross-resistance (resistance ratio, RR, was approximately 1.1) to the Akron strain of *An. gambiae*; the single compound displaying cross-resistance had an RR of 3.7. These results suggest that the *An. gambiae* potassium (Kv2) channel is an underutilized target for insecticide development. Furthermore, novel potassium channel blockers have the ability to mitigate resistant mosquitoes, as indicated by the low resistance ratios.

Education/Public Relations/ General

38 Vector biology and control in Iran: Challenges and opportunities

Ary Faraji, ary@slcmad.org, Seyed Hassan Moosa-Kazemi, Kamran Akbarzadeh, Javad Karimi, Mohammad Reza Fakoorziba, Mahmood Iranpour, Randy Gaugler

Malaria is the most important mosquito-borne disease in Iran, but sporadic transmissions of various arboviruses have also been documented. Leishmaniasis, transmitted by phleobotomine sandflies, is also of primary concern. The mosquito fauna in Iran currently includes 7 genera, 65 species, and three subspecies. However, the invasive Asian tiger mosquito, Aedes albopictus, has recently been documented to have established in southern Iran, near the Pakistan border. This is of particular concern because of the high rates of travel and dengue infections across these two international borders. Iran currently boasts a growing assemblage of entomologists that are being trained at local universities throughout the country in various public health and medical entomology programs. Additionally, there is a growing interest in integrated vector management strategies to combat vector-borne diseases throughout the country. Although residual insecticide spraying, use of insecticide treated nets, ultra-low volume aerosol spraying, use of larvivorous fish, and increase of biorational larvicide applications such as Bacillus thuringiensis israelensis and Bacillus sphaericus have increased in recent years, there still remains some gaps in technology and methods that would benefit from external training and involvement. The vector research and control community in Iran is eager to increase collaborations with international collaborators with the hopes of increasing our knowledge of vector biology, ecology, taxonomy, insecticide resistance, product and equipment assessments, surveillance evaluations, and training of future students and public health stewards.

39 "Dauntless Dottie" and the origins of mosquito control in the Pacific Northwest

Gordon Patterson, patterso@fit.edu

One hundred years ago Harrison Dyar began the study of mosquitoes in the Pacific Northwest. During the next twenty years the USDA dispatched George Bradley, Willard King, and Harry Stage to study the region's mosquitoes and to develop a plan for developing a mosquito control program. In 1946, a young Portland Commissioner named Dorothy

McCullough Lee made mosquito control an integral part of her advocacy for urban reform. Lee's two year campaign for reform and mosquito control culminated in 1948 when she was elected Portland's first female mayor. Lee's success in Portland is a testament to the importance of mobilizing public support for organized mosquito control.

40 Connecting with kindergartners through mosquito education

Eric Jackson, ericdj@leeschools.net, Brian Murphy, Neil Wilkinson

The Misunderstood Mosquito kindergarten program is one of the standards-based outreach programs offered by the Lee County Mosquito Control District to public and private elementary schools. It is designed to introduce primary-age students to life science by teaching the characteristics of mosquitoes and the basic attributes of insects. This science-readiness lesson provides kindergartners the opportunity to view living mosquito larvae, learn about where mosquitoes grow, and create their own mosquito models. Details of this highly-adaptable lesson will be presented as well as ways to customize it to meet individual district needs.

41 Best practices in educational outreach: Can an informational website effectively replace quality classroom-based education programs?

Shelly Redovan, redovan@lcmcd.org, Eric Jackson

Best Practices in Educational Outreach: Can an informational website effectively replace quality classroom-based education programs? Shelly S. Redovan, Eric Jackson, Wayne Gale The battle of balancing an annual budget for mosquito control programs can lead some program managers to consider reducing or replacing a contact-based education program, leaving the only education component of the agency's integrated pest management plan to be an informational website. The Lee County Mosquito Control District believes that a strong school-based educational outreach program is an important method in controlling mosquitoes and crucial to informing the public of the role they play in mosquito reduction. Educating the county's youth provides for a growing population of informed citizens that will share in the responsibility of controlling mosquitoes in their neighborhoods, resulting in lowered incidence of mosquito-borne disease and a more enjoyable outdoor experience for all residents. This presentation will discuss the strengths of school-based outreach programs and highlight some of the key weaknesses of taking a website-only approach.

42 Wing Beats: Cut, copy, paste, print

Stephen Sickerman, sickerman@comcast.net

Wing Beats magazine premiered 25 years ago as the official trade journal of the American Mosquito Control Association and the Florida Mosquito Control Association. Published quarterly, Wing Beats currently has a national circulation of almost 3800, with production and distribution costs supported by industry advertising. The processes and procedures for editing manuscripts and digitally designing and publishing each issue of Wing Beats will be discussed. Mosquito control professionals are encouraged to submit articles and photographs on every aspect of mosquito control: surveillance and control operations; new mosquitocides, equipment and technologies; vector behavior and biology; research, education and outreach; and environmental, legislative and regulatory issues.

43 A multi-faceted approach to community outreach

Rebecca Riley, rriley@hcphes.org, Sandra Kachur, Mustapha Debboun

Community education programs are designed to provide knowledge and facilitate voluntary behavior change. This is often a difficult task with limited resources, making community connections and innovative use of resources imperative. Over the last several years much has changed in cultural attitudes, increased demand for information, information distribution avenues, and program resources available. This presentation will provide an overview of the Harris County Public Health & Environmental Services Mosquito Control Division Community Education Program in Harris County, Texas and its use of community connections and multi-media to empower county residents and impact resident-initiated control activities.

Biology and Behavior II

47 Teasing apart the behavioral responses of *Culex tarsalis* to fish-associated semiochemicals in wind tunnel bioassays

Adena Why, awhy001@ucr.edu, Emerson Lacey, Ring Carde, William Walton

Culex tarsalis responds to the presence of fish-associated semiochemicals with a decrease in oviposition rate. This has been attributed to the presence of different classes of chemical compounds present in the water. In wind tunnel assays, we evaluated behavioral responses to isolated compounds, alone and in concert with Culex egg-raft pheromone, to determine their affect. We have tested three different compounds and our data indicate that Compound 1 acts as an attractant at long range.

48 Blood feeding habits of mosquitoes in Chicago

John-Paul Mutebi, grv0@cdc.gov, Linda Kothera, Joanie Kenney, Harry Savage

In 2012 blood-fed mosquitoes were collected in 7 different areas (Mt. Greenwood, Illinois Medical District, Trumbull Park, McKinley Park, Ogden Park, Portage Park, Winnemac Park) of the City of Chicago. The aim was to investigate blood feeding habits of mosquitoes within the City of Chicago especially West Nile virus vectors. A total of 839 blood-fed mosquitoes were collected and of these more than 99% had obtained blood meals from birds. The proportions of blood meals from different sources varied among regions but overall most blood meals were obtained from the American Robin (Turdus migratorius) (45%) and only 4 (0.5%) blood meals were obtained from humans.

49 Transcription sequencing in *Aedes aegypti* larvae exposed to pesticides

Liming Zhao, lmzhao@ufl.edu, Barry Alto, Dagne Duguma

Transcripton sequencing, or RNA-seq, has appeared recently as a powerful tool to gain a full picture of the expression profile of an organism, tissue or even a single cell. Aedes aegypti is the primer vector of dengue and chikungunya viruses that cause disease in humans. RNA-seq is used to understand the mechanisms of gene expression in Ae. aegypti larvae exposed to pesticides both VectoBac 12AS and Natular™ 2EC. We report on a study that documents transcriptional changes following exposure of pesticides during the larval stages in Ae. aegypti. A total of 15,045 transcripts were combined for comparisons of expression difference among two populations of Ae. aegypti and two pesticides currently in use to control this mosquito. The research final goal is to apply the molecular technology for laboratory and field trial studies, to reduce pesticide application while maintaining best mosquito control and environmental stewardship.

50 Evaluating the efficacy of household bleach wash solution as egg wash to increase survival rates in captive

Anita Schiller, ahowlett@hcp4.net

Producing Toxorhynchites rutilus (Tox), a U.S. native natural predator of Aedes albopictus and Ae. aegypti is an ongoing project at Harris County Precinct 4s DRAC/MAP program. With economics in mind, our program aims to streamline Tox. culture protocols to raise and release captive produced local phenotype Tox.. As large scale captive colonies experience fluctuating mortality rates, we evaluated the efficacy of various bleach solutions as egg surface wash in regards to overall survival rates from larvae to adults.

51 Exploiting the autogenous nature of *Aedes albopictus*

Don Shroyer, d.shroyer@irmosquito2.org

It has long been known that Aedes albopictus is facultatively autogenous in the initial ovarian cycle. That this characteristic is widespread and perhaps universal in geographic populations of the species deserves emphasis. Screening eight diverse geographic strains of Ae. albopictus reared on a rich larval diet revealed that 14 – 35% of females produced at least one autogenous egg, with average autogenous fecundity of 14 eggs per female. Moreover, frequency of autogeny is easily selected in the laboratory (up to 68%) and can be maintained indefinitely for many generations with careful husbandry. Autogenous strains of Ae. albopictus can be useful for certain experimental studies, such as investigations of vertical transmission of arboviruses. Autogenous strains are also potentially valuable to educational institutions or mosquito control agencies that need to maintain colonies without the complications associated with providing vertebrate blood meals.

52 Bite Back!

Julio Abreu, julio13abreu@gmail.com, James Forehand

Bite Back! is an innovative mosquito control device designed to trap mosquito larvae thus breaking the exponential growth cycle and can be easily introduced to work along side in the other existing Mosquito Integrated Control actions as per W.H.O Lab and Field Guide Lines.

The work done this time is simply a continuance of the Mosquito Integrated Control actions initiated back in 2013, in the same residential area of 436 single family homes and a neighboring commercial area occupying approximately 319,900 m², against *Aedes aegypti* as the dengue vector in Brazil, the results when Bite Back was deployed in conjunction with other control methods like larvicide treatments and residual and ULV space spray applications were again exceptional. As we can now, beyond doubt, declare that BiteBack indeed had played an important roll in controlling mosquito in the area as mentioned above with great consistence and effectiveness. As we are pleased to inform that again no cases of dengue transmission were reported while undertaking the treatment. At the same time, reported from the Health Department in Brasilia showed that at least 29 cases of dengue were confirmed in other residential areas near by.

Throughout 2014 last year, using BiteBack traps, we collected a total of 4.328 mosquito larvae, mostly from *Aedes aegypti* specie. This year, however, we had increased the number of traps placed in the area, from 19 traps as per last year to 30 this year. With the increment of traps, we have today, surpassed the number of captured larva in comparison with the same period as last year, as so far there are 5.503 larva captured and cataloged, which gives us an estimated number of 1.100.600 (average) of insects that did not return to the environment as an adult biting mosquitoes,

Last year, there were 865.600 less adult mosquitoes in the treated area due to the capturing of 4.328 larvas during that period. It is expected by the end of our work February next year to double this numbers.

In order to obtain optimum mosquito control, Bite Back should be applied in combination with the other control methods involved in Mosquito Integrated Control concept as per W.H.O Lab and Field Guide Lines. Bite Back trap is cost effective, simple and efficient, and it can also easily fit into the sustainability category, as it is uncomplicated to manage, environmentally safe, and uses no poison.

Latin American Student Competition and Symposium I

53 Evaluation of the toxic activity of plant extracts on *Triatoma pallidipennis*, an important vector in Chagas disease transmission

Laura Mayela Montes-Rincon, maye_bio@hotmail.com, Jesús Antonio Davila-Barboza, Ana Karen Leal-Olvera, Lucio Galaviz-Silva, Zinnia Judith Molina-Garza

Chagas disease is a zoonosis caused by flagellate protozoan Trypanosoma cruzi. Its drive chain involving a large numbers of vertebrate reservoirs and triatomine insect vectors that make impossible its eradication. In Mexico there's an annual incidence of 44,000 cases with a current prevalence estimated in 1,610,000 of people infected. The insecticide resistance in triatomine has been reported for deltamethrin, beta-cipermethrin, beta-cyfluthrin and lambda-cyhalothrin. To minimize the risk of resistance is necessary to develop other management strategies that will generate new chemical or biological alternatives to triatomine control. A viable alternative for the control of these insects emerged by the concept of integrated pest management that includes biological control, environmental management and the use of insecticides of biological origin, in which the risk of cross-resistance is reduced. In this work were performed bioassays using ethanolic extracts of 5 plants, the higher activity was observed with the use of the extract of Syzygium aromaticum, with a LC50 of 33mg/mL; followed by, Bougainvillea glabra with a LC50 43mg/mL; Lippia graveolens with LC50 58mg/mL; Ocimum basilicum with an LC50 of 65 mg/mL and Eucalyptus globulus with an LC50 of 97mg/mL.

54 Mutations kdr in the para-gene in Triatominae (Hemiptera: Reduviidae) vectors of Chagas disease in Mexico

Jesús Antonio Davila-Barboza, jesus.davilabr@uanl.edu.mx, Gustavo Ponce-García, Beatriz Lopez-Monroy, Irám Rodríguez-Sánchez, Pablo Manrique-Saide, Alejandro Villegas, Azael Che-Mendoza, Adriana Flores-Suarez

The transmission of Chagas disease is mainly through by triatomines.There are 19 species in Mexico of the 31 capable of transmitting the disease such as: Triatoma dimidiata, T. barberi and T. pallidipennis with intra domiciliary activity and T. longipennis, T. picturata and T. mazzottii with peridomestic activity. A wide range of methods for vector control have been tested in order to control domestic triatomine infestations, including biological control and pathogens as well as a number of physical and chemical methods including the use of pyrethroids. However, it has been reported the presence of resistance to this insecticides. We obtained DNA sequences encoding regions where mutations knockdown type (KDR) are reported. The comparison of these sequences showed high similarity with T. infestans.

55 **Expression levels of cytochrome P450 (CYP4C52v1, CYP68y3, CYP9K34, CYP9M10, CYP9J40 and CYP9AL1) determined by RT-qPCR in larvae of *Culex quinquefasciatus* exposed to different insecticides**

Irám Pablo Rodríguez-Sánchez, iramrodriguez@gmail.com, Olga Villanueva-Segura, Beatriz Lopez-Manroy, Michelle Zamudio-Osuna, Laura Martínez de Villarreal, Adriana Flores-Suárez, Jesús Villarreal-Pérez, Gustavo Ponce-García

In this Word we described the expression levels of seven transcripts of Culex quinquefasciatus to codified for P450 cytochromes, this measure by qPCR in larvae exposed to two permetrin insecticide concentrations (0.51 y 0.71 mg) compared with a control group without insecticide exposition, this specimens were collected from distinct places from northeaster of Mexico. Because the genes analyzed are not reported, it was necessary design oligonucleotide using traces sequences product of sequencing the genome Culex quinquefasciatus. With oligonucleotides designed and complementary DNA synthesized from total RNA from larvae were amplified by PCR messengers, then were proceeded to clone each product of these genes in from nine places analyzed were subsequently sequenced and these were compared to derive the percentages similarity. TaqMan probes were designed in conserved segments, and using the previously synthesized complementary DNA to deducted expression levels of each analyzed genes. We report the nucleotidic and amino acidic sequences of each from each population studied and levels of gene expression. We conclude that the analyzed genes are a powerful way to delve into the status of resistance that have developed wild populations of Culex quinquefasciatus in northeastern Mexico localities tool.

56 **The role of morphological and physiological traits in the acoustic behavior of *Aedes aegypti*: First step in the sound-based attraction technique in Colombia**

Hoover Pantoja, hoover.pantoja@gmail.com, Francisco Vargas, Ximena Bernal, Guillermo Rua, Horacio Cadena, Natalia Bedoya, Alejandro Vergara, Freddy Ruiz

The contradiction between immature indicators and dengue transmission reveals the need for effective techniques to collect adult mosquitoes. Recent studies have proposed the use of acoustic stimuli to capture mosquitos due to the relevance of acoustic signals in mosquito communication. However, to successfully design an acoustic trap to collect adult mosquitoes, complete understanding of the factors affecting the acoustic behavior of the mosquitoes is necessary. Here, we thoroughly characterize the signals of Aedes aegypti by investigating the role of morphological and physiological traits in its acoustic behavior. Recordings from 480 mosquitoes with different age, sexual status and size were analyzed and some of the main acoustic features of the courtship signals were extracted. However, only body size had a significant effect, with larger individuals producing wingbeats with significant lower dominant frequencies. Interaction between individuals of different size generated unusual relations between harmonics similar to those reported for Culex quinquefaciatus and Anopheles gambie but not previously described for Ae. aegypti. Although harmonic convergence often occurs between the second harmonic of the male and third harmonic of the female, when the female's fundamental frequency is close to the male's, harmonic convergence occurs between the third harmonic of the male with fourth harmonic of the female. This result suggests that, as proposed for other mosquito species, Ae. aegypti may rely on intermodulation for detecting conspecific signals. Therefore, we propose that stimuli used for Ae. aegypti attraction should focus on the distortion products generated by the interaction of frequencies rather than on pure tones.

57 **CHIKV in field populations of *Aedes aegypti* in San Marcos, Guerrero, México**

Martha Lopez, martha_a_a@hotmail.com, Karina Villanueva, Adriana Flores, Iram Sanchez, Gerardo Trujillo, Javier Valdes, Gustavo Ponce-García

CHIKV in field populations of Aedes aegypti in San Marcos, Guerrero, México.Chikungunya virus (CHIKV) is an arthropod-borne virus of the Alphavirus genus, which is transmitted to humans by Aedes spp. mosquitoes and was firstly identified in Tanzania in the mid 1950s. In December 2013, the World Health Organization reported the first local transmission of Chikungunya virus in the Americas with cases of indigenous origin from the Caribbean island of St. Martin. Since about 30 countries of the Caribbean have been reported with outbreaks of Chikungunya and local transmissions. In México the first local transmission case was reported in September 2014. In this study the presence of Chikungunya virus was determined in total of 23 mosquitoes' pools (male, female and larvae) of Aedes aegypti collected in San Marcos, Guerrero, Mexico. From 14 female pools 2 were positive for CHIKV, In 5 male pools 4 result positive and for larvae only one pool from 4 was positive to CHIKV. The virus detection was performed by using two different primers by the PCR and the results were confirmed by sequencing the positive samples.

58 First report of *Aedes aegypti* transmission of chikungunya virus in the Americas

Esteban Eduardo Díaz-González, esteban.diazgn@uanl.edu.mx, Tiffany Kautz, Alicia Dorantes-Delgado, Rose Langsjoen, Rubing Chen, Rosa Sánchez-Casas, Scott Weaver, Ildefonso Fernández-Salas

During a chikungunya fever outbreak in late 2014 in Chiapas, Mexico, entomovirological surveillance was performed to incriminate the vector(s). Seventy-five households in neighborhoods with suspected cases were sampled for mosquitoes, of which 80.0% (60) harbored Aedes aegypti and 2.7% (2) Ae. albopictus. A total of 1170 Ae. aegypti and 3 Ae. albopictus was collected and 81 pools were generated. Although none of the Ae. albopictus pools were chikungunya virus (CHIKV)-positive, 18 Ae. aegypti pools (22.8%) contained CHIKV, yielding an infection rate of 32.3 per 1000 mosquitoes. A lack of herd immunity in conjunction with high mosquito populations, poor vector control services in this region, and targeted collections in locations of human cases may explain the high infection rate in this vector. The inability of the Asian CHIKV genotype to adapt to Ae. albopictus coupled with the lack of known Ae. albopictus-adaptive mutations in our sequences, suggests that Ae. aegypti was the principal vector in the locations that we sampled, Ae. aegypti appears to be the principal vector of CHIKV in Southern Mexico, while the role of Ae. albopictus remains unknown.

59 The art state of Chikungunya virus (CHIK-V)

Olga Villanueva-Segura, karinaypwm@gmail.com, Beatriz Lopez-Manrroy, Gustavo Ponce-García, Gabriel Ruiz-Ayma, Laura E. Martínez de Villarreal, Adriana Flores-Suarez, Irám Pablo Rodríguez-Sanchez, Mayra A Gomez-Govea

Chikungunya virus is a member of the Alphavirus genus and the Togaviridae family isolated for the first time Newala district, Tanzani since then, Chikungunya´s fever has been expanded to other countries with several outbreaks. In America, the Chikungunya virus was reported for the first time in 2013, with a case reported in the Caribe and the number of cases has increased across the continent. In this research, we described the epidemiology and global expansion of CHIKV, the clinical features and the diagnosis in order to generate information to discuss priorities for further studies needed for effective disease control and prevention.The Alphavirus are enveloped particles and its genome is made of single stranded RNA (+) with ~12,000 nucleotides.The local transmission of Chikungunya virus has been identified in 17 countries, the Caribbean and South America, with 103,018 presumptive cases and 4,406 confirmed cases in these regions.The

infection of Chikungunya virus going through a period of silent incubation for 2 to 4 days. The initial clinical picture is abrupt, with high fever, headache, backache, myalgia and arthralgia. On the contrary, the lately phase is intense, affecting the tips mainly, specially the joints.There is no specific treatment, vaccine or preventive drug available for Chikungunya virus infection. The treatment is palliative, including rest, fluids and the use of analgesics and antipyretics. The best way to prevent the illness is to avoid the bite of the mosquito vector. There is the possibility that the virus continue expanding. A main efforts will considered in order to limit the virus transmission such a generation of epidemiological information and programs to control vectors.

60 Incidence and genomic diversity Chikungunya fever in Nuevo León, México

Irám Pablo Rodríguez-Sánchez, iramrodriguez@gmail.com, Michelle de Jesús Zamudio-Osuna, Mayra Alejandra Gómez-Govea, Adriana Elizabeth Flores-Suárez, Gustavo Ponce-García, Laura Elia Martínez de Villarreal, Jesús Zacarías Villarreal-Pérez

Chikungunya fever is a viral disease transmitted to humans by the Aedes aegypti and Aedes albopictus mosquitoes. It is characterized by sudden onset of fever usually accompanied by joint pain. The virus is transmitted from one person to another by the bite of infected female mosquitoes. According to WHO 2013 to March 2015 in the Americas, this disease is the cause of 26.391 confirmed cases and 184 deaths. Similarly, in Mexico according to PAHO 405 confirmed cases there. The diagnosis is carried out from total RNA and subsequent quantification by RT - qPCR. The aim of this study was to describe the epidemiology of chikungunya fever in Nuevo Leon within the period of 2014-2015. This was a retrospective study based on the data base of patients with clinical diagnosis of chikungunya (CHIK) that were referred to the State Public Health Laboratory of Nuevo León, México for diagnostic investigations from January 2014 to September of 2015. Our results indicate of 78 referred patients, a total of 13 (16.7%) patients were laboratory confirmed to have CHIK, and from this RNA samples were amplified by RT – PCR them cloning and sequencing to determines distinct genotypes. For all these reasons, CHIK is a public health problem in our country so it is vital monitoring and prevent this disease especially in the state of Nuevo León.

61 Larval indexes of *Aedes aegypti* (Diptera: Culicidae) and its relation to the occurrence of cases of Dengue and Chikungunya in the province of Orellana, Ecuador

Glenda Velásquez Serra, gvelasquez@inspi.gob.ec, Silvio Silva Salas, Jorge Luis Llangari Cujilema

The study of Aedes aegypti larvae fluctuations through entomological indicators (IE) define and redirect the actions of control. Such strategy, available in the program for surveillance and control of Ae. aegypti, allow reducing man-vector contact. Practical utility of the housing index (HI), deposit index (DI) and Breteau index (BI) with the occurrence of cases of Dengue and Chikungunya, is evaluated to determine monthly fluctuations and differences between three indexes in Orellana province, Ecuadorian Amazon. Methods: this was a descriptive, field and longitudinal design study. Indexes were obtained from censuses and interventions in home inspections to 100% of cases reported during EW 1-33, 2015. Entomological indexes were analyzed from averages and compared according cases, zones and months through analysis of variance, adopting significance level as "P" values less than 0.05. Results: By matching three indexes with dengue cases, difference was not statistically significant (P> 0.05). However, by linking CHIKV cases, it was observed that the fourth registered highest average in neighborhoods that reported cases of CHIKV 3-4; with a statistically significant difference (P <0.05). No statistical association between occurrence of cases found areas and indexes. Both BI and the

fourth highest average reached in April and lowest average in January, with a statistically significant difference (P <0.05). For its part, the DI recorded the highest average in May and lower average in January respectively; being a statistically significant correlation (P <0.05). The HI could be a good predictor to infestation by Ae. aegypti in cases of CHIKV. Keywords: assessment, entomological indices, Aedes aegypti, Dengue, Chikungunya

Student Competition III

62 Detection of CHIKV in field populations of *Aedes aegypti* in Guerrero State, Mexico

Martha Lopez, martha_a_a@hotmail.com, Karina Villanueva, Adriana Flores, Iram Sanchez, Gerardo Trujillo, Javier Valdez, Gustavo Ponce

CHIKV in field populations of Aedes aegypti in San Marcos, Guerrero, México.Chikungunya virus (CHIKV) is an arthropod-borne virus of the Alphavirus genus, which is transmitted to humans by Aedes spp. mosquitoes and was firstly identified in Tanzania in the mid 1950s. In December 2013, the World Health Organization reported the first local transmission of Chikungunya virus in the Americas with cases of indigenous origin from the Caribbean island of St. Martin. Since about 30 countries of the Caribbean have been reported with outbreaks of Chikungunya and local transmissions. In México the first local transmission case was reported in September 2014. In this study the presence of Chikungunya virus was determined in total of 23 mosquitoes' pools (male, female and larvae) of Aedes aegypti collected in San Marcos, Guerrero, Mexico. From 14 female pools 2 were positive for CHIKV, In 5 male pools 4 result positive and for larvae only one pool from 4 was positive to CHIKV. The virus detection was performed by using two different primers by the PCR and the results were confirmed by sequencing the positive samples.

63 Population Structure of *Aedes aegypti* in the Southeastern United States

Kristen Hopperstad, kahopper@ncsu.edu, Michael Reiskind

Aedes aegypti populations in the United States have declined since the invasion of Aedes albopictus in the 1980s. However, Ae. aegypti persists in some urban areas, and a resurgence of Ae. aegypti has been anecdotally reported in Florida and Louisiana. Mutation, genetic drift, and natural selection lead to genetic differentiation of local populations, and gene flow counteracts such differentiation. Thus, changes in gene flow have implications for the evolutionary trajectory of Ae. aegypti populations, possibly with concomitant changes in disease transmission potential. We characterized gene flow between populations of Ae. aegypti throughout the southern United States. Using 16 microsatellite markers, we genotyped 1,004 F0 and F1 adult Ae. aegypti mosquitoes from 23 localities. Results will be presented. Genotyping helps us better understand how mosquitoes move at a regional scale, and may offer the practical application of tracing emergent populations to their parent source.

64 Tests to reverse selection to pyrethoid resistance in *Aedes aegypti* populations from the South of Mexico

Farah Zamira Vera Maloof, zamirav@gmail.com, Connor Hendrich, Ashley Janich, Paisley Byrnes, Karla Saavedra-Rodriquez, William C. Black IV

For a decade pyrethroids have been used in Mexico to control the Dengue vector, Aedes aegypti. However, insecticide resistance has been developing in mosquito populations, jeopardizing the success of vector control programs to stop dispersion of the virus. The two main resistance mechanisms are the production of detoxification enzymes and point mutations in the

voltage-gated sodium channel (VGSC), which is the target of pyrethroids. The objective of this study is to evaluate the loss of pyrethroid resistant in field mosquitoes collections when released from pyrethroid pressure. We analyzed the enzymatic activity of oxidases, alpha and beta esterases, and glutathione-S-transferase (GST). Moreover, the frequencies of the two major kdr mutations (Ile1016 and Cys1534) in laboratory conditions were monitored without insecticide pressure. This study began with field resistance population from the south of Mexico. We monitored the frequency of enzymes and the kdr mutations under laboratory conditions with no exposure of insecticide. We analyzed eight population of mosquitoes: one from Chiapas, one from Guerrero and six populations from Yucatan. The frequencies of Ile1016 have a range of 0.36 to 0.94 and for Cys1534 the range goes from 0.70 to 1.0 α-Est activity has a range between 8.9 - 35.4 nmol/mg ptn/min, β-Est 2.3 - 15 nmol/mg ptn/min, oxidases with a range of 22.5 - 37.9 nmoles cit/mg ptn and GST of 3.12 - 19.54 mmoles/mg ptn/min. We predict that Ile1016 and Cys1534 will decrease as will the activity of detoxification enzymes.

65 **Host specific response to DEET selection in *Anopheles coluzzi* and *Aedes aegypti***
James Ricci, jricc001@ucr.edu, Bradley White

DEET (N,N-diethyl-3-methylbenzamide) is the most effective personal insect repellent currently in widespread use, and as such is essential in combating the spread of mosquito born disease both domestically and abroad. Despite its ubiquitous use and integral role in integrated vector management programs, the potential for the evolution of insensitivity to DEET in mosquito populations is not fully understood, and the mode of action of DEET is still debated. We demonstrate that insensitivity to DEET can be selected for in Anopheles coluzzi and Aedes aegypti populations, similarly to what has been demonstrated by others. We also demonstrate that insensitivity to DEET is host specific in Anopheles coluzzi and Aedes aegypti. DEET insensitivity selected for on one human volunteer does not translate to DEET insensitivity on another human volunteer. This suggests that DEET in some way acts in synergy with host-specific odorants that vary due to diet, microbiome, and/or other factors. Based on these findings, it is unlikely that insensitivity to DEET can be selected for in wild mosquito populations.

67 **Is resistance accumulating in Louisiana's mosquitoes? Deciphering the susceptibility status of *Culex quinquefasciatus* to three larvicides**
Nicholas DeLisi, ndelisi55@gmail.com, Kristen Healy

The susceptibility status of Culex quinquefasciatus to many larvicides is underrepresented in Louisiana. The objective of this research is to determine whether the susceptibility of Cx. quinquefasciatus to Bacillus sphaericus, methoprene, and spinosad has diminished over time. Mosquitoes are being collected from across the state and undergo larval bioassays that elucidate changes in susceptibility. Results will shed light upon the efficacy of current control procedures, and will contribute to better utilization of larvicides.

Backyard Spraying: The Pest Management Professionals Perspective Symposium

68 **Introduction and goals of the backyard spraying symposium**
Janet McAllister, jvm6@cdc.gov

The availability of area wide mosquito control is not universal in the United States. Regardless of whether there is an organized program, individual citizens are more and more frequently opting to

hire a pest management professional (PMP) to provide mosquito control services. While the AMCA is widely recognized as an organization to turn for expertise on area wide control of mosquitoes, there is little interaction between the PMP and AMCA . This symposia is intended to educate AMCA members on the business of mosquito control from the PMP viewpoint.

69 Available products in mosquito control

Ron Harrison, rharriso@orkin.com

Community wide mosquito control was employed 20 to 30 years ago. Increased concern of the impact pesticides have on the environment, community wide mosquito control has reduced leading to the emergence of pest control companies, individuals and property owners involvement in mosquito control. Products available for mosquito control are targeted at larva and adults. Larva are targeted using mechanical and physical control as well as IGRs and bacteria. Adults are controlled with a variety of products ranging from sugar to classical insecticides such as pyrethroids. The use of classical insecticides are lethal to adults if they spend enough time where products are applied. Products are impacted by environmental conditions. This often requires application of products more often than quarterly and every other month to insure mosquito control. Adding IGRs to mosquito adulticides appears to reduce mosquito populations.

70 Best Management Practices

Brian Forschler, bfor@uga.edu, Tiffany Nguyen

The object of backyard spraying for mosquito management is a relatively new concept that has been questioned in regard to determining efficacy. In urban entomology the definition of efficacious outcome is critical for understanding an efficacious outcome to a pest management program. Our work using a vacuum-sampling device in residential neighborhoods in Georgia has shown that approximately 30% of homes have quantifiable mosquito populations. The same percentage of homes treated by specific technicians show the same proportion of infestation. This survey data couple with our work measuring mosquito populations before and after pesticide spraying at sites under our control as well as residual data from foliage samples taken at measured time intervals after application indicate that source reduction and treatment of larval breeding sites is critical to efficacious backyard spraying programs. Therefore any backyard spray program should have a component associated with affecting larval populations not simply relying on contact and residual activity of the spray.

71 Individual accounts and spraying for communities contracting mosquito management for municipalities and communities

Mike Swan, t.swan@ufl.edu, Patrick Prather

Due to the lack of organized private mosquito management beyond backyard control programs, a need was identified for a greater range of mosquito management services. Municipal Mosquito was created to scale into mosquito management service offerings not readily available to a geographic area with any dedicated mosquito control district. Municipal Mosquito has offered Integrated Mosquito Management services to private neighborhoods, management companies, private organizations, and municipal accounts for over nine years. These services consist of not only barrier sprays and misting systems, which are convenient and efficient for both residential clients and businesses, but also larvacide programs, mosquito surveillance, mosquito identification and speciation, real time RTPCR disease testing, and adult mosquito management. The scope of these types of mosquito management services can begin to test the resources of most

traditional pest service companies. Resources to qualify for larger commercial and municipal clients may require certain quantities of equipment, mandatory degrees/certifications, very stringent insurance coverage, and program knowledge of large scale mosquito management. Structural pest companies often look to mosquito control as a seasonal offering, but the investment in dedicated personnel and their education and training will define the effectiveness of your mosquito department. The successful shift into large scale mosquito management requires a focus much different than residential and commercial pest control. This shift is towards the needs of municipal clients and the service offerings necessary to anticipate and meet the changing wants, needs, and expectations of the citizens or community.

Adult Control III/New Tech and Non-Targets

72 What can you do with 100 million transgenic mosquitoes?

Derric Nimmo, derric.nimmo@oxitec.com, Andrew McKemey, Camilla Beech

Mosquito-borne diseases, such as dengue fever, chikungunya and malaria, are major and increasing international public health concerns. The two main vectors of dengue are Aedes aegypti and Aedes albopictus and current control measures are proving difficult against both these mosquitoes. In an Oxitec control programme transgenic male mosquitoes (male mosquitoes do not bite or transmit disease) are released continually over a wide area to mate with the target pest population; progeny from these matings die and the target population declines. Over 100 million genetically engineered Oxitec male mosquitoes have been released over the past 5 years in trial around the world. We will present about what this has achieved and future prospects around the world.

73 Aerial release of sterile/treated male mosquitoes to combat disease

Ralph Breslauer, rsb5779@gmail.com, Hanan Lepek

As sterile, treated and modified mosquito techniques, continue to mature and show tremendous efficacy in field trials, the need to distribute very large quantities of these mosquitoes becomes greatly needed. Reaching hard to get to areas or large infected (population) centers is difficult and potentially risky to do by truck and by other current approaches. We will be describing a full offering that includes storing and releasing large quantities of treated mosquitoes safely from the air as part of a comprehensive mosquito control program. Break through technology has just come to market that keeps these mosquitoes safe while being released from fast moving aircraft to cover large tracts of land. Whether the size of the area to be treated is large or there exists a hesitation to generally spray chemicals for mosquito control, using the aerial release of treated mosquitoes, can be the solution. They are the 'smart bombs' of the treatment world, seeking out and targeting female mosquitoes wherever they may hide at any time of day or night. The urgent need to address Malaria, Dengue, Chikungunya, EEE, West Nile virus etc. demands a large scale effective treatment plan that this new technology can now address.

74 Optimization of production and quality control for *Aedes albopictus* males for inundative field release

Hanano Yamada, hanano78@hotmail.com, James Mains, Corey Brelsfoard, Stephen Dobson

The mass production of highly competitive insects is essential to autocidal control strategies such as Sterile Insect Technique and Incompatible Insect Technique (Wolbachia). The fundamental goal is to optimize rearing techniques to achieve high numbers of the insect and to produce males that are reproductively competitive relative to wild type males, while minimizing costs. We have

adapted existing protocols and are formulating novel tactics for the mass production of Aedes albopictus males, with the goal of inundating indigenous populations and reducing populations of this invasive and medically important mosquito. In this presentation, I will provide an overview of the biological quality of an Ae. albopictus strain that is artificially infected with the wPip Wolbachia type from Culex pipiens.

75 Male mosquitoes as delivery vehicles for insecticide

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

Mosquito control remains the only effective means available to combat many mosquito-borne diseases and to reduce populations of nuisance mosquito species. Chemical insecticides used to control mosquitoes are typically in the form of larvicides and adulticides, sprayed by hand or by vehicles, e.g., truck- or aircraft-mounted sprayers. Larvicides have been proven effective at reducing mosquito-borne disease transmission. However, its implementation at a 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 find and treat (i.e., 'cryptic breeding sites'). Here, we will present results of an auto-dissemination approach in which mosquitoes 'self-deliver' a pesticide to cryptic breeding sites. The results of laboratory and field experiments examine for impacts of pyriproxyfen (PPF) directly applied to adult male Ae. albopictus and Ae. aegypti including: (1) the ability of PPF-treated males to cross-contaminate females and to (2) directly deliver PPF to breeding sites. Data demonstrates that males can contaminate both female adults and oviposition containers in field cage tests, at levels that eliminate immature survivorship. Field trial results from 2014 and 2015 with Ae. albopictus demonstrate an ability of PPF-treated males to transmit lethal doses to introduced oviposition containers, and a decline in the Ae. albopictus population was observed following the introduction of PPF-treated males.

76 ADAM, Auto-Dissemination Augmented with Males: A novel strategy to control *Aedes aegypti*

Jodi Holeman, jholeman@mosquitobuzz.net, Katherine Ramirez, James Mains, Corey Brelsfoard, Stephen Dobson, Anthony Cornel, Steve Mulligan

The recent invasion of Aedes aegypti L. into several, dispersed areas of California, beginning in 2013, has created significant public health issues for mosquito and vector control agencies. A primary vector for dengue and chikungunya viruses, this mosquito is also closely associated with human habitation for oviposition sites and exhibits a preference for humans as hosts. In invaded neighborhoods, Ae. aegypti is a huge biting nuisance and source of resident complaints, yet it is difficult to control utilizing conventional treatment methods. Thus innovative approaches are called for in combating this important vector. During 2015, the University of California, Davis and the Consolidated Mosquito Abatement District collaborated with MosquitoMate, Inc., to evaluate their novel control strategy, auto-dissemination augmented by males (ADAM), against Ae. aegypti within a small neighborhood of Clovis, Fresno County, California. ADAM incorporates the mass rearing and release of large numbers of male mosquitoes that have been dusted with the insect developmental inhibitor, pyriproxyfen (ppf). After release, dusted males mate with local females and transfer ppf to the females. PPF contaminated females and released males visit and transfer the insecticide to oviposition and larval development sites where the material disrupts immature development. We will discuss the elements and issues involved in development of the study; including the selection of study sites, gaining access and acceptance from homeowners, development of procedures and protocols specific to the evaluation of ADAM against Ae. aegypti in an arid habitat, as well as dealing with media and providing and disseminating public education materials.

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

James Mains, jmains@mosquitomate.com, Hanano Yamada, Corey Brelsfoard, Robert Rose, Stephen Dobson

The Asian tiger mosquito (Aedes albopictus) is an aggressive daytime biter and a public health concern due to its ability to transmit medically important pathogens (e.g., dengue, chikungunya). This invasive species has colonized much of the U.S.A., despite intensive use of pesticides, 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 the classical, sterile insect technique, the Wolbachia approach is based on the release of non-biting, male mosquitoes, to effectively sterilize the targeted population. On July 26, 2013, MosquitoMate Inc. was granted an EPA Experimental Use Permit (No. 89668-EUP-1) to conduct field performance testing of this approach for the Asian tiger mosquito in the continental U.SA. Presented here are the results of 2014 and 2015 field trials designed to examine for Wolbachia infected male Ae. albopictus performance under field conditions and for an impact on the targeted population.

78 ***Wolbachia pipientis* infected male mosquito release pilot program to control *Aedes albopictus* in Los Angeles County**

Susanne Kluh, skluh@glacvcd.org, Harold Morales, Tanya Posey, Angela Brisco, J. Wakoli Wekesa, James W. Mains, Corey Brelsfoard, Stephen L. Dobson

Since the re-detection of Aedes albopictus in Los Angeles County in September of 2011, this invasive mosquito species has continuously spread throughout the San Gabriel Valley and into other portions of the Los Angeles Basin despite intensive traditional control efforts by both Greater Los Angeles County (GLACVCD) and San Gabriel Valley (SGVMVCD) Vector Control Districts. The pilot program in collaboration with researchers from the University of Kentucky and MosquitoMate is designed to examine releases of Wolbachia pipientis infected male mosquitoes (ZAP strain). Wolbachia is a naturally occurring bacterium that can stop egg development in Ae. albopictus and other mosquitoes. Questions include: 1) ZAP male survival and fitness following cross-country shipment, 2) persistence and spread of males within an EPA-defined release zone, 3) effect on egg hatch rate, due to mating with local wild-type, and 4) unintended establishment of the ZAP-type Wolbachia within the introduction zone. The outcome of the pilot program will help determine a decision about a larger scale release program intended to control and eliminate Ae. albopictus populations. To make this project possible, regulatory issues had to be addressed and the local community had to be informed to ensure their support. Release as well as control sites were chosen based on previous surveillance records and releases were conducted between May and October 2015 in the city of South El Monte. This presentation describes the regulatory, outreach, and surveillance procedures for releasing sterilizing males into an isolated population of Ae. albopictus.

79 **Wildlife Lighting Certification Program: Protecting wildlife through responsible lighting practices**

Michael Hudon, m.hudon@irmosquito2.org

Infrequent low use rate mosquito control adulticide applications are criticized by some members of the public that fear collateral damage to non-target insects. These people and others may unwittingly be doing far more damage to positively phototaxic insects and other non-targets by

their use of artificial night lighting 365 days a year. This talk will focus on Wildlife Lighting Certification and other ways to mitigate those effects.

Disease Vector I/ Surveillance

80 Development and field evaluation of the Sentinel Mosquito Arbovirus Capture Kit (SMACK)

Brian Johnson, brian.johnson@jcu.edu.au, Tim Kerlin, Sonja Hall-Mendelin, Andrew van den Hurk, Stephen Doggett, Cheryl Toi, Ken Fall, Scott Ritchie

Although sentinel animals continue to be used to monitor arbovirus activity, inherent ethical and serological issues necessitate the need for viable alternatives. Here, we present the development of the passive sentinel mosquito arbovirus capture kit (SMACK) that enables the detection of arboviruses on honey-baited nucleic acid preservation cards (FTA®, Whatman®) and has a similar trap efficacy as standard light traps. Trap efficacy was compared against CDC miniature (standard and ultraviolet) and Encephalitis vector survey (EVS) light traps in field trials conducted in northern Australia. The ability of the SMACK to detect arbovirus activity was assessed in comparison to Passive Box Traps (PBT) in the Cairns region and individually in the remote Northern Peninsula Area (NPA) of Australia during the 2014 and 2015 wet seasons, respectively. Trap efficacy of the SMACK was comparable to both CDC light traps (mean capture ratio 0.86: 1) and was consistently greater than the EVS trap (capture ratio 2.28: 1) when CO2 was supplied by either a gas cylinder (500ml/min) or dry ice (1 kg). During the 2014 wet season, the SMACK captured significantly (t_6=2.1, P= 0.04) more mosquitoes than the PBT, and 2 and 1 FTA® cards were positive for Ross River virus and Barmah Forest virus, respectively, while no arboviruses were detected in PBTs. During 2015, arbovirus activity was detected at all three NPA sites and ca. 27% of FTA® cards tested positive for either Murray Valley encephalitis virus (2 detections), West Nile virus (Kunjin subtype; 13 detections), or both viruses (on two occasions). These results demonstrate that the SMACK is a simple and effective passive arbovirus surveillance tool that may also be used as a traditional overnight mosquito trap.

81 Honey-card surveillance of arboviruses in Florida: Can we beat the chicken?

Nathan Burkett-Cadena, nburkettcadena@ufl.edu, Thomas Unnasch, Jennifer Gibson, Rui-de Xue, James McNelly, Miranda Lauth, Alberto VanOlphen

Early detection of arbovirus transmission is the best available tool for protecting the public against mosquito-borne pathogens, giving vector control districts a critical temporal window of action to impact the vector population. Sentinel chickens are currently the most widely used method of arbovirus early detection in Florida. However, technical time-lags and logistical difficulties can reduce their early-detection value. Honey-card surveillance, in which trapped mosquitoes are mass-screened through their salivation onto honey-soaked nucleic acid preserving substrates, was developed as a surveillance tool in remote areas of Australia, but has the potential for widespread application in other regions. We compared honey-card method of arbovirus surveillance with sentinel chickens for detection of arbovirus transmission in St. Johns and Volusia Counties, FL, USA. Three distinct traps, an updraft light trap, a gravid trap and an automated resting trap, each utilizing the honey-card method of arbovirus surveillance, were operated in the vicinity (10-30m) of five sentinel flocks in each county. Traps used programmable timers to regulate the hours of operation so that each could operate seven days per week with only two site visits each week. Eastern equine encephalitis virus (EEE) and West Nile virus (WNV) were detected from both honey-cards (RT-PCR) and sentinel chickens (serology). The number of arbovirus detections using honey cards was quantitatively and temporally similar to detections by

sentinel chickens for EEE, but not WNV. Variation in mosquito species composition and honey consumption among traps likely contribute to the effectiveness of this surveillance method for various arboviruses.

82 Mosquito pools? How do they fit? Early arbovirus detection in Panama City Beach, Florida

Michael Riles, michael.riles@comcast.net, James Clauson

Early detection of the presence of West Nile Virus occurred in Panama City Beach, FL. Six out of nine mosquito pools of Culex quinquefasciatus were found positive for West Nile virus (WNV). An arbovirus advisory was put into place for Bay County, FL at that time. Based on Florida Department of Health Guidelines; positive mosquito pools are not generally used to determine advisories and alerts; only sentinel chickens. How do we incorporate positive mosquito pools into the operational utility of determining advisories and alerts?

83 Laboratory evaluation of the rapid analyte measurement platform (RAMP) dengue virus assay

Kristen Burkhalter, ktb3@cdc.gov, Harry Savage

We present sensitivity and specificity laboratory evaluations of the Rapid Analyte Measurement Platform (RAMP) Dengue Virus (DENV) assay, which is designed to universally detect all 4 serotypes of DENV in mosquito pools. The RAMP DENV assay was able to detect all 4 DENV serotypes (DENV 1-4) in virus-only samples and mosquito pools spiked with virus. The RAMP DENV assay also detected DENV 1-4 in mosquito pools containing a single mosquito intrathoracically inoculated with one of the 4 serotypes and 24 laboratory-reared negative mosquitoes. No false positives were detected in negative mosquito pools or in samples containing high titers of non-target arboviruses.

84 Variation in species composition and diversity of mosquitoes relative to arbovirus activity in Lowndes County, Georgia

Mark Blackmore, mblackmo@valdosta.edu, Elizabeth Ann Broadie, Jamaica L. Hill, J. Erin Parker

Surveillance for vectors of West Nile virus and Eastern Equine Encephalitis virus has been conducted in south-central Georgia since 2001. Mosquito populations were sampled using CDC miniature light traps baited with dry ice and Reiter gravid traps baited with hay infusion at 16 locations in Valdosta and surrounding areas of Lowndes County, Georgia. Species composition at sites where these arboviruses have been repeatedly isolated from mosquitoes will be compared to that of sampling sites in which virus activity historically has been low or absent. Temporal variation between sites and site categories (high virus/low virus) also will be examined.

85 Mosquito control and arboviral surveillance in Harris County and the City of Houston, Texas

Mustapha Debboun, mdebboun@hcphes.org, Martin Reyna Nava, Rebecca Riley, James Dennett, Kyle Flatt, Umair Shah

Harris County (HC) which includes the City of Houston is a region that is populated with competent mosquito vectors that have a high potential for transmitting arboviruses such as West Nile virus, St. Louis Encephalitis, Dengue, Chikungunya and other viruses in this region. This presentation will provide an overview of the rigorous surveillance and integrated mosquito

management of mosquitoes in the 3rd most populous county in the nation and the 4th largest city in the United States by the Mosquito Control Division of Harris County Public Health and Environmental Services.

86 Update on mosquito research in the Entomology Department at U.S. Naval Medical Research Unit 6, Peru

Craig Stoops, crgstps@gmail.com, Gissella Vasquez, Victor Lopez-Sifuentes, Carmen Flores-Mendoza

The Entomology Department at the U.S. Naval Medical Research Unit No. 6 conducts research on vectors of malaria, dengue, chikungunya and their ecology, biology, taxonomy, control and surveillance. In addition, we conduct research on sand flies, ticks and other medically important arthropods across the diverse ecological regions found in South America. We test and develop products that are used to protect deployed U.S. military forces while transferring technology to our host nation, Peru, and other regional partners. Recently, a colony of Anopheles darlingi was established and is being used to produce Plasmodium vivax sporozoites for malaria vaccine candidates as well as product testing and behavioral experiments. In March 2016, NAMRU-6 plans to open a new state of the art insectary in Iquitos for rearing multiple mosquito species with the capability of conducting experiments with both infected and uninfected mosquitoes. Our personnel also continue to build on NAMRU-6's 30-year history and provide updated vector distribution information for assessing vector-borne disease risk and training to regional partners to improve medical entomology capacity throughout South America.

87 Estimating current and future distribution of malaria and its primary vectors in northern South America based on climatic and anthropogenic changes

Temitope Alimi, t.alimi@umiami.edu, Douglas Fuller, Whitney Qualls, Socrates Herrera, Myriam Arevalo-Herrera, Martha Quinones, Marcus Lacerda, Jon Beier

Changes in land use and land cover (LULC) as well as climate are likely to affect the geographic distribution of malaria vectors and parasites in the coming decades. At present, malaria transmission is concentrated mainly in the Amazon basin where extensive agriculture, mining, and logging activities have resulted in changes to local and regional hydrology, massive loss of forest cover, and increased contact between malaria vectors and hosts. Employing presence-only records, bioclimatic, topographic, hydrologic, LULC and human population data, we modeled the distribution of malaria and two of its dominant vectors, Anopheles darlingi, and Anopheles nuneztovari s.l. in northern South America using the species distribution modeling platform Maxent. Results from our land change modeling indicate that about 70,000 km2 of forest land would be lost by 2050 and 78,000km2 by 2070 compared to 2010. The Maxent model predicted zones of relatively high habitat suitability for malaria and the vectors mainly within the Amazon and along coastlines. While areas with malaria are expected to decrease in line with current downward trends, both vectors are predicted to experience range expansions in the future. Elevation, annual precipitation and temperature were influential in all models both current and future. Human population mostly affected An. darlingi distribution while LULC changes influenced An. nuneztovari s.l. distribution. As the region tackles the challenge of malaria elimination, investigations such as this could be useful for planning and management purposes and aid in predicting and addressing potential impediments to elimination.

88 Diversifying with dengue-the RAMP ® platform

Paolo Lobo, plobo@responsebio.com, Adriana Cajiao, Crystal Selluski, Pamela Tan, Angela Carter

Mosquito-borne infectious diseases have been a global problem for many years. Many arbovirus infections, including West Nile and Dengue fever, have no vaccines and limited treatment options. Consequently, the most successful prevention methods have involved control of the vectors that can carry a disease-causing virus. Hence, viral detection in these vectors is of utmost importance in the active surveillance and monitoring of vector populations in affected areas. The RAMP® system provides easy-to-use, high-sensitivity tests that can reliably and efficiently detect West Nile Virus (WNV) and now Dengue Virus (DENV) antigen in mosquito populations. The RAMP® Dengue test is an immunochromatographic assay indicated for use as an in vitro diagnostic product to quantitatively determine the presence of dengue viral antigens in mosquito homogenates. The RAMP® Dengue test was evaluated for analytical reactivity and sensitivity, hook effect, and cross-reactivity. The test was found to detect antigen of all four Dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) in mosquito homogenate, showed no evidence of high dose hook effect up to four times the upper limit of the reportable range, and no evidence of cross-reactivity with WNV.

Backyard Spraying: The Pest Management Professionals Perspective Symposium

89 One bird, two stones. How MADs and backyard operators can co-exist for a common cause

Joey Osborne, osborne.joey@gmail.com

For many years MADs and similar entities bore the burden of mosquito control. As a result, these institutions firmly ingrained into the world of mosquito control their modus operandi. The systems and protocol that came from years of R&D, trial and error testing, etc., served the public well and are largely responsible for the relatively low volume of mosquito vectored disease that we know today in the US. As funding decreased in combination with ever-increasing technological advancement , the private sector began to step in and fill gaps in mosquito control that had either always existed or came about as a result of cutbacks. The result of this disruptive shift is a booming, entrepreneurial-based business model that, though indirectly, and maybe not even to its awareness, shares much of the same mission as the MADs and others that were its predecessors. The private sector mosquito control industry has many advantages over its public sector counterparts. Things like private funding, less restrictive regulation, entrepreneurial innovation tendency are advantages that should continue to lead to growth and innovation in the field of mosquito control. In summary, the current evolution in mosquito control from the perspective of service providers has potential to enhance the level of control, which should be the primary objective of all concerned parties.

90 Business model for individualized mosquito control

Heather Gordy, hgordy@arrowext.com

Due to recent attention on mosquito-borne diseases, more and more residential and commercial pest control customers are interested in individualized mosquito control. In the pest management industry, customer retention is critical to the firms sustainability. Not addressing a customer's mosquito control concerns may open opportunity to competitors. While many pest management companies are already operating successful mosquito programs in this market, others are evaluating their business models to determine if developing a program of their own is a good fit. This discussion will cover many of the things pest management owners and operators must consider before starting or restructuring a mosquito control program. These include;

equipment, product efficacy, environmental impacts, service frequency, government regulation, licensing, training and more.

91 Training needs for pest management professionals

Claudia Riegel, criegel@nola.gov, Janet McAllister

Backyard barrier treatments for the use of mosquito control has become a popular offering conducted by pest control and specialized mosquito control companies. A variety of treatments are available and are usually applied one time or on a 30 or 60 day cycle. Several levels of training for employees, managers, and owners of these companies are available. Each state will have the minimum requirements needed to obtain and maintain appropriate licenses to operate. The number of continuing education credits required will vary by state and training may be obtained in educational sessions at trade shows, association meetings, or at conferences. Some companies also have internal technical support and have structured training above the minimum requirements of the state. However, training programs offered specifically by mosquito control professionals that are geared to this industry are limited. Professionals that provide backyard mosquito control would highly benefit from courses that teach mosquito identification, bionomics, and the principles of integrated mosquito management. Individuals specializing in mosquito control associated with mosquito control associations, universities, and county/parish mosquito control programs would be excellent candidates to provide high level programs targeted to the pest industry.

92 The use of backyard treatments by mosquito control districts for routine and targeted mosquito control

Brendan Carter, bhcarter@nola.gov, Erin Cloherty, Sarah Michaels, Claudia Riegel

Mosquito control districts (MCDs) utilize multiple strategies to control mosquitoes including educational campaigns, source reduction, and ground & aerial adulticiding and larviciding. Treatments of individual properties may also be utilized if manpower and resources allow. Residential yards can be treated using ultralow volume equipment or with mist blowers that produce larger droplets. Treatments may also have residual insecticide activity, depending on the method of application and active ingredient. Residential treatments may reduce the number of adult mosquitoes experienced in individual yards and can be conducted in areas at risk of arbovirus transmission. With the threat of mosquito-borne diseases such as chikungunya, dengue and West Nile virus in an area, the use of backyard treatments may be a viable tool to reduce adult vector mosquito populations including Aedes spp. and Culex spp. The presentation will discuss case studies from New Orleans where the majority of vector species rest outdoors and commonly breed in containers in an urban environment.

Latin American Symposium II

93 Diet on *Aedes aegypti* larval stage as a determining factor in the tolerance of insecticides

Abdiel Martin-Park, abdielmp@colostate.edu, Beatriz Lopez-Monroy, Maria Torres-Sepúlveda, Gustavo Ponce-García, Consuelo Ruiz-Herrera, Laura Martinez de Villarreal, William Black IV, Iram Rodriguez-Sanchez

Aedes aegypti is a predominantly urban vector, utilizing the abundance of artificial containers as larval sites and feeding almost exclusively on humans. It's a primary vector of dengue and chikungunya viruses. Since there is no specific vaccine or drug available, vector control is the

only option. Neurotoxic insecticides, particularly organophosphate and pyrethroids, are the most frequently used compounds against larvae and adults. The use of insecticides is being challenged by the rapid emergence of resistance. Insecticide resistance is known to be energetically costly, resulted in stressed out mosquitos with altered metabolism. Larval nutrition is an important key for adult developmental parameters and tolerance against insecticides. Metabolites as free amino acids and acylcarnitines circulating in the blood can be used for diagnosis for metabolic illness and imbalances. The metabolic/energetic cost in larvae, and how it affects the adult's tolerance to insecticides is still unknown. Eggs from the field were hatched and fed with three different diets: 1) high in protein, 2) high in lipids and 3) balanced. Third instars larvae were tasted with temephos, malathion, and permethrin. The baseline toxicity to insecticides was determined using the standard susceptible New Orleans strain. By tandem mass spectrometry we obtained the reference ranges concentrations of amino acid, acylcarnitines and organic acids. The means and standard deviations were determined and statistically significant differences were observed between groups and diets. Ranges of acylcarnitines, amino acid and organic acids from the two strains (susceptible and resistant) and diets were also determined and significant differences were found.

94 Evaluation of organic and inorganic substrates for mass production of parasitic nematodes of mosquito larvae

Rafael Pérez Pacheco, rafaelperezpacheco@yahoo.com, Alicia Alonso Ramos, Dalila Pérez Pablo, Edward Platzer

For mass production of parasitic nematodes of mosquito larvae river sand is normally used as a substrate of the culture medium, but the weight and the characteristics of the sand movement generated during transportation reaching affect yield of cultures 80 -90%. We evaluate organic and inorganic substrates (two species of moss Oaxaca and one species of the Republic of Chile, to replace the use of sand in the culture media. For production of Romanomermis culicivorax and Romanomermis iyengari, in different experiments were set up in polyethylene containers, 100 females and 100 males in each culture medium or 1 g of nematodes in each medium. After 4, 6, 8, 10 weeks, cultures were flooded with distilled water to induce hatching and then determine the number of nematodes obtained in each experimental unit. In R. culicivorax, the cultures of 100 females and 100 males at 8 weeks pre parasitic producing on sand was 38,300 and in Chile moss 33,000, and 16 weeks in sand the yield was 32,000 and 64,000 in moss and in cultures with 1g at 8 weeks in sand the production was 53,200 and in moss 46.320 and on 16 weeks 46,000 in sand, 40,000 in muss from Chile and 42,000 in muss of Oaxaca. In the production of R. iyengari in the cultures of 100 females and 100 males in sand 25.375 pre parasitic nematodes were recorded and 16.625 in moss Oaxaca, and in the cultures with 1g of nematodes at four weeks 237.750 nematodes in sand and 200,000 in moss Chile. The nematodes pre parasites collected in the different experiments were used to be the infection of larvae of Culex quinquefasciatus and the mosquito larvae were parasitized efficiently, it indicated that the substrates do not affect the infectivity capacity of nematodes

95 Metabolites of *Aedes aegypti* larval stage in the tolerance of spinosad

Irám Pablo Rodríguez-Sánchez, iramrodriguez@gmail.com, José Alfonso Flores-Leal, Olga Karina Villanueva-Segura, Grifith Lizarraga, Alfredo Ignacio Córdova-Galván

Aedes aegypti is a primary vector for various arboviral infections including dengue and chikungunya virus. Since there is no specific vaccine or drug available for the treatment of these virus, vector control is the only options to reduce the spread of these infections. Insecticides organophosphate and pyrethroids, are the most frequently used compounds against larvae and

adults. However, the use of insecticides is being challenged by the rapid emergence of resistance. On the other hand, the naturally derived insecticide spinosad have shown a low environmental and human risks; also offers approaches to integrated pest management and insecticide resistance management. Interestingly, the metabolites such as free amino acids and acylcarnitines circulating in the hemolymph can be used for detection for imbalances. However, the metabolites in larvae, and how they distributed with the presence of spinosad is still unknown. Ae. aegypti eggs from the field were hatched and fed with diets balanced. Bioassay consisted of a minimum of six concentrations of spinosad, at least two replicates per concentration, and six control replicates (ethanol alone). The baseline toxicity of Ae. aegypti to insecticides was determined using the standard susceptible New Orleans strain. After 24h, mortality was recorded. By tandem mass spectrometry we obtained the reference ranges concentrations of amino acid, acylcarnitines and organic acids. The means and standard deviations were determined and statistically significant differences were observed between spinosad concentrations. Ranges of acylcarnitines, amino acid and organic acids from the two strains (susceptible and field) were also determined and significant differences were found.

96 Deltamethrin resistance in *Aedes aegypti* (Diptera: Culicidae) from Mexico

Yamili Contreras-Perera, yamjaz_85@hotmail.com, Gustavo Ponce-García, Beatriz Lopez-Monroy, Olga Villanueva-Segura, Valentin Uc, Azael Che-Mendoza, Pablo Manrique-Saide, Adriana E. Flores- Suarez

The mosquito Aedes aegypti (L.) is the primary vector of dengue and Chikungunya viruses in Mexico. In Mexico, vector control relies on the application of insecticide, however, the insecticide resistance is a serious problem for worldwide vector control programs. In this study, the susceptibility status to technical grade insecticide deltamethrin was determined using CDC bottle bioassay on five natural populations of Aedes aegypti from Mexico. We determined the presence and frequency of kdr V1016I and F1534C mutations. The results revealed high resistance levels in all populations to deltamethrin, with resistance ratios of 133, 18, 6, 32 and 25 fold from populations of Merida, Progreso, Hunucma, Hoctun and Agua Dulce, in Yucatan and Veracruz southeast of Mexico . The frequency of kdr V1016I mutation were (0.77, 0.66, 0.45, 0.34 and 0. 56) and for F1534C were (1.00, 0.97, 0.64, 0. 59 and 1.00) from the same populations described above. The kdr-F1534C mutation was fixed in two populations suggesting its potential role in deltamethrin resistance.

97 Effect of mating in the cuticular hydrocarbons of female and males of *Aedes aegypti* (Diptera: Culicidae)

Samantha D. Ruiz-Aguirre, samdav2185@hotmail.com, Leopoldo Cruz-López, Gabriel Fuentes-Maldonado, Ildefonso Fernandez-Salas, Armando Ulloa

The cuticular hydrocarbons of females and males Aedes aegypti (L.) mosquitoes were analyzed before and after they mated in laboratory conditions. Six cuticular hydrocarbon n-pentacosane, n-hexacosane, n-heptacosane, n-octacosane, n-nonacosane and n-hentriacontane were the most prevalent in both. These cuticular hydrocarbons were observed principally in the mated stage in both sexes, where the proportion of n-heptacosane was higher. These results show the effect of mating in the profile of cuticular hydrocarbon in both sexes of Aedes aegypti.

98 Measurement of aerially sprayed droplets through a canopy to recognize cloud and spray dispersion in common mosquito resting sites

Grifith Lizarraga, glizarraga@clarke.com, Jesse Julien

ULV aerial applications and their measurement have been a topic of debate since their inception. As technology moves forward and ULV applications are well-established spraying techniques, there is a greater need for methods that can measure and compare sprayed clouds through unique habitats and environments. This study was designed to measure how droplets from an aerially sprayed product behave throughout and when they hit the inside of a canopy at different heights. A pole with droplet impingers (spinners) installed at different elevations collected the dispersion of the cloud as it moved vertically and weather stations monitored microclimates within the canopy. This study will help improve proficiency of an aerial spray and how droplet density and droplet size will be filtered through a canopy and other areas that mosquitoes are both resting and active; it will guide and delineate how much product will, potentially, be suspended in locations that are difficult to penetrate.

99 Mosquito records from Mexico VII: The mosquitoes of Queretaro State

Aldo Ortega, agrortega@hotmail.com, Adelfo Sánchez, Félix Ordóñez, Quetzaly Siller, Ramón Méndez

Introduction: The knowledge of the Culicidae fauna occurring in Queretaro state of Mexico is very poor. There are a few historical records in very old studies and most of them are dificult of access for consult. Objective: Identify the diferent species of Culicidae mosquitoes that occurs in all physiogeographical regions of Queretaro State, Mexico. Methods: Mosquitoes of they all life stages were collected in all available water body: ponds, swamps, arroyo sides, artificial containers, tree holes, rock holes, bromeliad axils, and landing/biting over the collecting personnel. Most immature stages were put live in individual vials for rearing and collect the associated exuvia and adult stage; some immature were killed with hot wáter and preserved in vials with 90% etanol. Adult stages were killed with triethylamine vapors and preserved in dry vials. All specimens were transported to the laboratory (UAAAN-UL) for mounting and identification. Results: A total of 2059 specimes were collected (622 females, 357 males, 897 larvae, and 183 pupae). Two subfamilies: Anophelinae and Culicinae, 5 Tribes (Aedini, Culicini, Culisetini, Sabethini, and Toxorhinchitini), 15 genera, 16 subgenera and 41 species were collected. 24 species are new records for Queretaro state.

100 Ticks infesting some domestic animals in Tapachula, Chiapas, Mexico

Noe Lopez-Lopez, neto.nel@hotmail.com, Jorge Torres-Manzon, Jose A. Juarez-Ordaz, Sergio E Bermudez, Armando Ulloa

We presented a preliminary checklist of tick collected from domestic animals (dog, horse, cow), human, and by flagging vegetation at sites in Tapachula, Chiapas, in the southern Mexico. Ticks were collected and preserved in 70% ethanol. All specimens were examined and species and sex was determined following the taxonomic criteria of Fairchild et al. (1966) for adult's ticks. Three tick's species were identified: Rhipicephalus sanguineus s.l. (Latreille), Rhipicephalus (Boophilus) microplus (Canestrini), and Amblyomma cajennense s.l (Fabricius).

101 Anthropophilic biting activity of *Kerteszia* species in a malaria endemic region of department of Tolima in Colombia.

Jesus Eduardo Escobar, jeescobar@unisalle.edu.co, Ranulfo Gonzalez, Martha Quinones

The foci of Cunday-Villarica in the department of Tolima in Colombia, has presented cases of malaria for over thirty years and has been the area that provides 70% of the cases presented in the department. Control measures in the area by the Ministry of Health in the late eighties, caused a significant drop in malaria cases. However, since 1984 has not done entomological monitoring.

This is worrying, given that the region is still considered by the ministry of health as an area at risk of acquiring malaria, with poor access to health services. The only entomological study area by Quiñones et al. (1984) found the species An. lepidotus (currently as An. pholidotus) as the most abundant (99.5%) and highly exophagic behavior. The other species found, An.boliviensis presented very low density (0.5%). The aim of this study was to determine the biting behavior of the Kerteszia species in the foci of Cunday-Villarica. 2,487 female of Anopheles were caught landing in humans. Adult females were identified by morphological keys and confirmed with molecular markers. Samplings in peak hours and for 24 continuous hours were made. Of all females captured, 1,781 (71.7%) corresponded to An. pholidotus and 706 (28.3%) belonged to An. boliviensis. An. pholidotus and An. boliviensis showed diurnal activity, with a major peak between 17-18 h. An. pholidotus showed a smaller peak of activity at 11 h. in intradomicile, and another at 6 h. in outdoors. Both species showed preferences biting outdoors, a situation that complicates their control through traditional vector control measures aimed primarily at endophagic species. Consequently, the use of personal protection is recommended.

102 Potential resistance mechanisms (organophosphates, carbamates and pyrethroids) in *Triatoma dimidiata* Coast-Pacific genotype from Mexico

Arturo Acero Sandoval, navio81@hotmail.com, Américo Rodríguez Ramírez, Teresa López Ordóñez, J. Genaro Ordóñez González, Francisco Solís-Santoyo, Patricia Penilla-Navarro

Insecticide resistance mechanisms and susceptibility levels were determined in Triatoma dimidiata Pacific-Coast genotype of Mexico. Susceptibility tests and biochemical assays were performed for Cytochrome P450 content, α and β esterases as well as those para nitrophenyl acetate-based esterases (ρ-NPA), and glutathione-s-transferase (GST); all were quantified per mg of protein in first instar bugs of different age in days. The letal concentration (LC50) obtained with malathion was 43.83 µg/cm2, 4.71 µg/cm2 with propoxur and 5.80 µg/cm2with deltamethrin. Esterases with the three substrates tested, and GSTs showed higher activity in 17-18 days old bugs as compared to those of 2-3 days old. No difference in the content of cytochrome P450 between individuals of the same stage was found. The election of the insecticide for T. dimidiata control must be based on the insecticide resistance population status and the possible mechanisms involved.

Student Competition IV

104 The oviposition of *Aedes albopictus* in response to Copepoda in field conditions

Jimmie Teague, jlteague@uncg.edu, Gideon Wasserberg

Biological control methods can play an important role in the prevention of vector-borne diseases. To be effective, the control agent should act as bait or not be repellant. The copepod Mesocyclops longisetus has been used as a control agent for mosquito larvae. However, the ability of female mosquitoes to detect and respond to its presence in terms of oviposition site selection is poorly understood. In this study, we used Aedes albopictus and assessed ovipositional site response and its effect on hatching rates in the presence of the predator, Copepoda. We hypothesized that the presence of the predator will have a negative effect on the oviposition, as well as on the hatching rates. If the location for possible oviposition is the habitat of a natural predator, then the mosquito will show preference for another location. We conducted this experiment by establishing 4, 100-meter transects, each containing a set of mosquito egg collecting cups, in Peabody Park in Greensboro, NC. These oviposition traps were randomly placed in pairs of treatment and control. The treatment cups contained various densities of Copepoda, either 50 or 100, and the control cups contained distilled water. The cups were deployed in 5-day intervals, then collected and

analyzed. The results showed that there was not a significant difference in oviposition between cups. Nor was there an effect on the hatching rates in the presence of a predator. These results showed promise with the gravid females not responding to a foreign enemy. This illustrated a positive relationship in population control of nuisance agents by the use of natural predators. By passively controlling and reducing the population of vector species, the risk of several mosquito-borne diseases can be mitigated.

105 Modeling mosquito population dynamics using surveillance, treatment, and climate data in Tarrant County

Daniel Dawson, dan.dawson@ttu.edu, Nina Dacko, Christopher Salice

Quantitative relationships between mosquito control and mosquito populations are challenging to establish. Mathematical modeling is a valuable tool that can help characterize and apply these links for vector control purposes. In this study, we use generalized linear modeling to model population dynamics of Culex quinquefasciatus in Tarrant County, Texas in response to climate, habitat, and treatment variables. Preliminary analyses show that of these variables, temperature is the most important factor influencing population dynamics.

106 Toxicity and physiological action of basic amines to mosquito and cockroach

Minyuan Tie, mtie@ufl.edu, Baonan Sun, Maia Tsikolia, Ulrich Bernier, Jeffrey Bloomquist

The yellow fever mosquito, Aedes aegypti, is a primary vector of dengue virus, and a major target of insecticide and repellent design and development. In the present work, 1-methylpiperazine and the related basic amines, 1-methyl-pyrrolidine and triethylamine (a commonly used anesthetic in the study of Drosophila melanogaster and mosquitoes) were analyzed for their lethal and electrophysiological actions. Topical assay of 1-methylpyrrolidine and glass contact assay of 1-methylpiperazine on adult Aedes aegypti showed low toxicity (22% mortality at 5 ug/mg body weight and zero mortality at 25 ug/cm2, respectively). Extracellular recordings of nerve firing activity showed that all three basic amines increased the discharge frequency of mechanoreceptor neurons located in the American cockroach (Periplaneta americana) tarsus. When applied topically at a dose of 30 micrograms, nerve firing increased significantly compared to the acetone control group ($n = 5$, $p < 0.001$). Patch clamp experiments showed inhibition of voltage gated potassium channels in the human neuroblastoma SY5Y cell line and a cell line expressing the Anopheles gambiae Kv2.1channel. N-methylpyrrolidine and 1-methylpiperazine blocked potassium channels with IC50s in the micromolar range, with less inhibition of sodium channel current (SY5Y cells). Exposure to these compounds is usually in the vapor phase. Potential alternation of Aedes blood pH by inhaled organic bases will be identified in the whole insect, and compared with the neural activity profile.

107 Mosquito surveillance in the Tongatapu Island Group, Kingdom of Tonga

Tom Swan, Mike@municipalmosquito.com, Jon Harding, Milen Marinov

In Tonga, both dengue and chikungunya fevers cause numerous deaths each year. An outbreak of chikungunya virus in April 2014 infected 10,000 Tongan residents, resulting in numerous fatalities. Surprisingly few mosquito studies have been conducted in Tonga, leading to a paucity of information on Tongan mosquito species. We sampled mosquito larvae at 88 sites throughout the Tongatapu Island Group in December 2013. Water chemistry measurements (temperature, pH, dissolved oxygen, conductivity), habitat type, and GPS points were also recorded. Nine species of mosquitoes were collected, including the invasive mosquito Aedes albopictus. This is only the second known collection of this mosquito in Tonga since its first published record in

2012. Aedes albopictus, Ae. aegypti, and Culex quinquefasciatus were the most commonly occurring species. Aedes albopictus co-occurred with the introduced species Cx. quinquefasciatus, Ae. aegypti, and the endemic Stegomyia, Ae. tongae. Larvae of the collected species favored artificial habitats, with abandoned car tires being the most common container habitat in which they were found. From this research, a pictorial identification key for mosquito larvae has been produced to help the Tongan Ministry of Health. Increasing public awareness and the reduction of known mosquito habitats may aid in decreasing dengue and chikungunya incidence within the Kingdom of Tonga.

Poster Session Abstracts

Adult Control

P-01 The behavior of *Anopheles gambiae* s.I. on bendiocarb powder treated electrostatic net in eave tubes: Observations using a simple novel video surveillance system suited for the field.

Michael Cordel, michael.cordel@biogents.com, Issa Lyimo, Ladslaus Mnyone, Kija Ng'habi, Andreas Rose, Sergius Sperling

The growing resistance to the insecticides used in insecticide-treated bednets (ITNs) and Indoor Residual Spraying (IRS) increasingly constrains the control of malaria vectors. Recently developed electrostatically charged surfaces can bind and efficiently transfer powdered insecticides to landing mosquitoes. Electrostatic nets with insecticides induced a high mortality even in resistant strains. They are used in the so-called Eave Tube, a mosquito contamination device developed by the MCD consortium. Eave tubes are installed in the upper walls of human habitations, after the eaves have been closed and the windows have been screened. As part of the proof of concept, we observed the behaviour of mosquitoes on insecticide-powdered electrostatic nets in the Eave tubes, using a simple Video Surveillance System (VSS). Trials were carried out in the semi-field and field in Tanzania. The VSS consists of a low cost action camera and a battery-operated red LED lighting system. Experiments were carried out with An. arabiensis in an experimental hut under semi-field condition, where we compared Bendiocarb-powdered to untreated electrostatic nets, and in human habitations in the field, where we observed the behaviour of wild mosquito populations on Bendiocarb-powdered electrostatic nets only. Video recordings were analysed using the software Ethovision XT 11. In the semi-field, contact times of Anopheles arabiensis were significantly higher on Bendiocarb-powdered nets, compared to untreated nets. In 94,2% of the cases, they were long enough to ensure the transfer of a lethal dose. Under field conditions, contact times of wild mosquitoes trying to enter the houses through the Eave Tubes were even longer, with 90,5% being longer than 5 s.

P-02 Implementing the CDC bottle bioassay for pesticide resistance testing of *Culex pipiens* and *Cx. tarsalis* in Placer County

Jessica Stevenson, jessicas@placermosquito.org, Mary Sorensen

Jessica A. Stevenson and Mary A. Sorensen The CDC time-response bottle bioassay (Brogdon and Chan) adapted from Brogdon and McAllister (1998) is a surveillance tool for detecting resistance to insecticides in vector populations. Insecticide resistance in two West Nile virus vectors, Culex tarsalis and Cx. pipiens in Placer County is a growing concern. In 2014, a resistance testing program was implemented to describe the geographic distribution and severity of resistance to adult mosquito control products. Twelve populations were tested, and during the testing many variability issues were detected. Over time fourteen variables were identified and

three investigated so that they could be better controlled to ensure reliable and replicable results in future resistance testing. Variables were separated into two categories; biological and methodological. Biological variables included mosquito physical fitness, genetic variability, age variability, sex variability and handling variability. Age variability was investigated to determine whether the age of an adult mosquito affected its susceptibility to a pesticide and the range of time response curves within a susceptible population was investigated. Methodological variables included bottle cleaning, pesticide coating and dosage variability. Bottle cleaning methods were investigated to reduce the risk of pesticide contamination. The CDC bottle bioassay was adapted accordingly. The results of these studies will help other mosquito and vector control agencies produce accurate resistance data.

P-03 Activity patterns of *Aedes albopictus* within a diverse environment of residential and agricultural use and introduction of a new slow-release pyriproxyfen formulation for controlling wild vector populations

Xavier Castells Sierra, javier.castellssierra@gmail.com, Casey Parker, Roberto Pereira, Philip Koehler, Alexandra Chaskopoulou

Aedes albopictus, also known as the Asian tiger mosquito, is known to flourish in a variety of habitats including residential and agricultural areas. In 2014 and 2015, a small-scale surveillance and control program was initiated targeting Ae. albopictus populations within the premises of an educational agricultural farm, a 45-ha diverse field environment of residential and agricultural use. Activity patterns of wild mosquito populations were monitored to create baseline activity data using 3 different surveillance methodologies: standard ovitraps, CDC light traps baited with dry ice, and BG Sentinel traps. Aquatic habitats were identified and monitored for the presence of mosquito larvae. In 2015, a novel slow-release pyriproxifen formulation was introduced in all detected breeding sides within the residential zone of the farm, whereas surveillance continued in both agricultural and residential zones using the most efficient surveillance methodology (ovitraps). Activity levels of Ae. albopictus were compared between the treated and untreated zones across both years. A significant decrease of vector populations was observed in the treated zone for up to 3 weeks post treatment indicating that this novel pyriproxifen formulation has potential to be used for vector control in medium-size residential environments as part of a broader integrated vector control approach.

P-04 Residual effectiveness of lambda-cyhalothrin on *Aedes albopictus* in Virginia

Benjamin McMillan, benm93@gmail.com, Jake Bova, Sally Paulson

Aedes albopictus (Skuse) is the most invasive mosquito in the world and a competent vector for many viruses. Because Ae. albopictus is active during the day, standard mosquito control efforts utilizing spray trucks to administer insecticides offer little control, as this method is generally directed towards crepuscular species. This species is a major biting pest in suburban yards and may transmit viruses such as Chikungunya, thus homeowners have increasingly searched for methods to control the mosquito. Residual pesticides applied to mosquito resting sites in vegetation have been shown to reduce pest mosquito populations. We tested five plants common in landscaped areas (Rhododendron spp., Thuja spp., Buxus spp., Lonicera spp., and Miscanthus spp.) of Virginia for the residual efficacy of lambda-cyhalothrin (Demand CS, Syngenta, Greensboro, NC) against Ae. albopictus. Plants were sprayed with lambda-cyhalothrin in concentrations of 0.06% or 0.03% using a Stihl sr200 mist blower just to the point of runoff. Care was taken to ensure the tops and undersides of the leaves were treated. Plants were kept outside on a 20 ft. sq. plot and exposed to natural environmental conditions and were watered as necessary. Female mosquitoes of two-three d of age were placed into glass vials with leaves from

the treated plants and were evaluated for their condition after one h and 24 h of exposure. Testing was done at d zero and weeks one through eight. Knockdown percentages were collected at one h after exposure, and mortality percentages were collected after 24 h of exposure. Both knockdown and mortality was highest for the d zero and the week one results across all plant species tested, but overall residual efficacy varied by plant species.

P-05 Field evaluations of residual pesticide applications and misting system on militarily relevant materials against medically important mosquitoes in Thailand

Arissara Pongsiri, arissarap@afrims.org, Alongkot Ponlawat, Kijchalao, Kijchalao, Seth Britch, Kenneth Linthicum

A key strategy to reduce insect-borne disease is to reduce contact between disease vectors and hosts. In the current study, residual pesticide application and misting system were applied on militarily relevant materials and evaluated against medically important mosquitoes. Field evaluations were carried out in Chanthaburi province, Thailand from February to July 2015. Groups of ground-mounted structure of HESCO geotextile (2x3 m) and camouflage netting (3x3 m) were constructed. These materials are extensively used for perimeter defense during US military field operation. The residual pesticide formulation containing λ-cyhalothrin and the pesticide misting system containing permethrin were applied on each type of structures. To evaluate effects of residual treatment and misting system, day and night biting mosquitoes were collected using BG sentinel traps and CDC light traps, respectively. Results demonstrated that treated HESCOs and camouflage netting enclosures substantially reduced mosquitoes (≥50%) compared to untreated units. Moreover, misters significantly reduced mosquito populations at treated units more than treated structures without misters. The effect of residual pesticide on materials lasted for more than two weeks but could not be retained to week ten after application in this tropical climate. Residual pesticide applications and misting system on militarily relevant materials revealed the promising results for mosquito control in Thailand.

P-06 Characterization of *Culex tarsalis and Cx. quinquefasciatus* (Diptera: Culicidae) response variability to pyrethroid and organophosphate insecticides may improve interpretation of CDC bottle bioassay results

Mary Sorensen, marys@placermosquito.org, Jessica Stevenson

While conducting bottle bioassays with local populations of mosquitoes as well as susceptible laboratory strains, it was noticed that results from populations tested more than once were variable, raising the question of how dissimilar the bioassay responses of two populations needed to be in order to indicate resistance. In this study, laboratory colonies of Culex tarsalis and Cx. quinqeufasciatus known to be susceptible to pyrethroid and organophosphate insecticides were repeatedly subjected to the CDC bottle bioassay with etofenprox and naled, with the aim of understanding the expected variability in response to the bottle bioassay within a population. The information from this study may improve bioassay design by informing the number of replicates needed for assays to be reproducible, and should allow more precise determination of when bioassay results demonstrate resistance.

P-07 Operational aspects of the CDC Autocidal Gravid Ovitrap

Veronica Acevedo-Soto, gra3@cdc.gov, Manuel Amador, Gilberto Félix, Roberto Barrera

Dengue viruses cause hundreds of millions of infections every year in tropical and subtropical countries. Unfortunately, there is not a single universal vector control method capable of suppressing Aedes aegypti (L.) populations. Amongst novel control tools or approaches are

various types of traps targeting gravid females or their eggs. Most assessments of the newer traps have concentrated on their effectiveness to monitor or control mosquitoes, but little information has been provided on aspects of their operational use. Here, we provide details of the operational use of the Centers for Disease Control and Prevention Autocidal Gravid Ovitrap (CDC-AGO trap) for the surveillance and control of Ae. aegypti. We placed three AGO control traps per premise in two relatively isolated neighborhoods and two additional neighborhoods were monitored as reference sites without control traps. Between March 2013 and April 2015 we serviced the AGO traps fourteen times in each community (every two months). Common trap problems encountered were: absent or broken trap tops (1- 1.5%), flooded (0.1 - 0.7%) or dry (0.5 - 1.3%), and missing (0.3 - 0.8%) or vandalized (0.5 - 1.4%) traps. Most traps kept a volume of infusion between 45- 97% of its original volume (10 L). A detailed account of non-target organisms captured in AGO traps has not yet been made, but in addition to mosquitoes, we captured other insects such as flies, ants, cockroaches, grasshoppers, butterflies, dragonflies, bees, and lizards. Trap coverage ranged between 83-87% of houses in both communities throughout the study. We interpret such high levels of trap retention over time as an expression of acceptance from the community.

Behavior/Biology

P-08 The effects of La Crosse virus infection on repellent response in *Aedes albopictus* and *Aedes triseriatus*

Kevin Chan, kchan90@vt.edu, Sally Paulson, Carlyle Brewster

La Crosse virus (Family Bunyaviridae, California serogroup, LACV) is the most common and important endemic mosquito-borne virus of children in the United States. The principal vector of LACV is Aedes triseriatus (Say); however, Ae. albopictus has also been shown to be a competent vector for LACV. Because there are no vaccines for most mosquito-borne viruses, repellents are recommended as an important line of protection from infection for individuals. However, recent studies have shown that Ae. aegypti mosquitoes infected with Sindbis virus were less affected by DEET. The purpose of this study was to determine if La Crosse virus-infected Ae. triseriatus and Ae. albopictus will demonstrate an altered blood-feeding response in the presence of DEET. LACV-infected and uninfected mosquitoes were given a blood-membrane that was either treated with 5% DEET or untreated. The number of mosquitoes that probed and blood-fed from the membrane were recorded for 30 minutes. Results showed that uninfected mosquitoes fed normally from the untreated membrane and neither probed nor blood-fed from the DEET-treated membrane. The LACV-infected mosquitoes also fed normally from the untreated membrane, but some individuals also probed and fed from the DEET-treated membrane.

P-09 Spermathecal lobe usage in *Aedes aegypti* and *Aedes albopictus*

Carrie De Jesus, cedejesus@pacbell.net, Michael Reiskind

Aedes aegypti and Ae. albopictus are important vectors of dengue and chickgunya. Both species share similar behavioral and physiological traits including a three lobed spermathecae, the sperm storage unit. The spermatheca has a large medial lobe and 2 smaller paired lateral lobes. Females are capable of controlling the entrance and exit of sperm from each lobe, suggesting the potential for asymmetrical usage of different lobes. In this investigation we examined if females selected sperm from the medial or lateral lobe. We also examined how male body size influenced lobe selection and egg laying. Small and large males were reared by altering larval density, females were reared separately. Females were mated with either small or large males and then provided blood meals. After each gonotrophic cycle 10 females were sacrificed, and the number of eggs

laid and sperm in each spermathecal lobe were counted for up to four cycles. We detected a decrease in the number of sperm from both medial and lateral lobes in Ae. aegypti after the second gonotrophic cycle. However we did not detect sperm depletion in Ae. albopictus. Aedes albopictus stored more sperm and laid more eggs than Ae. aegypti. Females that mated with larger males in both species laid more eggs compared to smaller males. In conclusion we did not detect a preference towards the medial or lateral lobe. We did find the females that mated with large males produced more eggs, which suggests a previously unreported role of male size in determining female fecundity.

P-10 *Aedes aegypti* surveillance in Fresno County 2013-2015

Jodi Holeman, jholeman@mosquitobuzz.net, Steve Mulligan, Charlie Smith, Katherine Ramirez

Following the initial detection of Aedes aegypti in Fresno County during the summer of 2013, the Consolidated Mosquito Abatement District designed a surveillance grid to effectively monitor its distribution and spread, using the fishnet tool in the geographic information system (GIS) ArcMap ESRI®. The grid has been reestablished each year since 2013 and expanded based Ae. aegypti detections. In 2013, the area encompassed approximately 16.5 km2 in the City of Clovis and was divided equally into a 10 by 10 grid with 100 cells (each cell 0.16 km2). An ovitrap was placed within each cell as a fixed site for ongoing monitoring throughout the season. During 2014, the grid was expanded by 780 meters both to the north and to the west of the 2013 detections for a total grid size of 23.6 km2. Further spread of Aedes aegypti in 2015 required expansion of grid to cover an area of 58.6 km2. Ovitraps were used as the primary surveillance tool to monitor the spread of Aedes aegypti within this community. In addition to the ovitraps, BG Sentinel traps as well as CDCAGO traps were used to monitor population abundance. A summary of the surveillance data collected from 2013-2015 will be presented.

P-11 Attraction of phlebotomine sandflies (Diptera: Psychodidae) to modified CDC light traps with light-emitting diodes of different colors

Jorge Jesus Rodriguez-Rojas, jorge.rdz3288@hotmail.com, Wilfredo Arque-Chunga, Ildefonso Fernández- Salas, Eduardo A. Rebollar- Téllez

The objective of the present study was to evaluate and to compare Centers for Disease Control and Prevention (CDC) modified light traps with light-emitting diodes (LED) blue, white, red and green lights against standard incandescent lights for the collection of sandflies species. We conducted field evaluations using a randomized-block design in a tropical forest of southern Mexico over an annual cycle. In 720 night-traps, we collected 914 specimens (78% female and 22% male) of 13 species of two genera (Lutzomyia and Brumptomyia). The 27.8% of all sandflies were collected with incandescent light traps and there was significant difference ($H = 4$, $df = 14.58$, $p = 0.0057$) in the mean number of sandflies caught with the five different traps. Analyzing catches by species per trap, we found that out of thirteen species sandflies caught with blue, white, green and incandescent light, only two species were more abundant Lutzomyia steatopyga and Lutzomyia deleoni. But with red LED, ten species were caught and two species were more abundant Brumptomyia mesai (26.9%) and Lutzomyia steatopyga (26.2%). Incandescent light trap attracted more specimens known as vectors of Leishmania spp. such as Lutzomyia cruciata, Lutzomyia shannoni and Lutzomyia ovallesi. Our prototype LED-modified traps operated well in a tropical environment and power consumption is less than used incandescent light, as well as, LED-modified traps are others choices to help vector surveillance programs.

P-12 Nondestructive DNA extraction for vouchering mosquitoes and sand flies

Ioannis Giantsis, igiant@afs.edu.gr, Alexandra Chaskopoulou, Marie Claude Bon

Classical DNA extraction methods of insects require crushing of the entire sample, or removing one single appendage with the risk to lose the remaining ones, precluding deposition of the sample as a voucher. Nondestructive techniques allow the isolation of genomic DNA, without damaging the morphological features of the specimens. Such techniques, although are available for numerous insect groups, have not been applied on any member of the medically important families of mosquitoes (Diptera: Culicidae) and Phlebomonine sand flies (Diptera: Psychodidae). This study presents the first nondestructive DNA extraction methodology for vouchering taxa of mosquitoes and sand flies, which provided sufficient amounts of DNA, tested in a verified barcode (cytochrome oxidase I gene), while preserving their morphological integrity. Application of this method in sand flies, allowed successful insect identification post DNA extraction, since all basic structures necessary for identification (pharynx, cybarium, genitalia) remained intact. The development of the methodology was more challenging in mosquitoes, due to the sensitivity of key morphological characters (scales, color). A small modification in the method achieved the extraction of sufficient DNA quantity, while preserving the integument of the mosquitoes, although a small proportion of the scales and the color still appeared to have been lost. In addition to the usability and efficiency (minimum insect handling and time-saving procedures) of our methodology, preserving of the original insect specimen post DNA extraction is highly advantageous as it allows for a) insect utilization for further analysis and b) insect storage for future morphological verification.

Disease/Vector Studies

P-13 Ecology of West Nile fever across four European countries: History of WNV transmission, vector population dynamics & vector control response

Alexandra Chaskopoulou, achaskopoulou@ars-ebcl.org, Gregory L'Ambert, Duscan Petric, Romeo Bellini, Thomas Groen, Lawrence Marrama, Dominique Bicout

West Nile virus (WNV) is endemic in Europe at least since the 1960s when human and equine infections were reported from many countries. Human outbreaks have occurred in southern France and Spain in the 1960s, Belarus and southern Ukraine in the 1970s, western Ukraine in 1985, and southeastern Romania in 1996. The Romanian WNV outbreak resulted in >500 clinical cases and 17 deaths. Until 2004, only lineage 1 and 3 WNV strains had been found in Europe. For the first time a lineage 2 strain caused a major WNV epidemic in 2010 in Greece with 262 clinical human cases and 35 fatalities. Since then, WNV lineage 2 outbreaks have been reported in many European countries. Understanding the interaction of ecological factors (biotic and abiotic) that affect WNV transmission is crucial for preventing or decreasing the impact of future epidemics. The synchronous co-location of competent mosquito vectors, virus, bird reservoir hosts, and susceptible humans (biotic factors) is necessary for the initiation and propagation of an epidemic. Weather is the key abiotic factor influencing majorly the biological life cycles of the mosquito vector, the virus, the reservoir host and the interactions between them. The purpose of this paper is to describe and compare mosquito population dynamics, weather conditions, and WNV virus transmission in 3 ecologically different environments (urban, rural, agricultural) across 4 European countries (Italy, France, Greece, Serbia) with ongoing outbreaks. Vector control strategies against WNV epidemics will be reviewed as well. Improving our understanding of WNV ecology is a prerequisite step for appraising and optimizing vector control strategies in Europe with the ultimate goal to minimize the probability of WNV infection.

P-14 Validity of a novel morphological character to distinguish female *Culex restuans* and *Culex pipiens* collected from gravid traps

Brian Byrd, bdbyrd@wcu.edu, Bruce Harrison, Tyler McKinnish, Kevin Caillouet, Michael Hutchinson, Ryan Harrison, Yanju Li, Cody Dunlap

Culex restuans Theobald and Culex pipiens L. are important enzootic vectors of West Nile virus in the Northeastern and Mid-Atlantic regions of the United States. In these regions, Culex pipiens is the primary vector of West Nile virus to humans. Thus, many organized mosquito control districts use gravid traps to collect both species to establish entomologic metrics as a primary surveillance method to assist in the prediction and reduction of human disease. Although long-recognized as a challenge to distinguish morphologically, the adult females of these two species are commonly identified by microscopic examination of the external morphology. Inaccurate identification, or pooling both species together, yields ambiguous results that confound the interpretation of virus positive pools. Here we describe a novel morphological character that readily and reliably distinguishes the adult females of Cx. restuans and Cx. pipiens. Culex restuans has uniformly black erect scales on the vertex and/or occiput whereas Cx. pipiens has brown erect scales with several light tan or pale erect forked scales generally near the midline of the head on the vertex and/or occiput. The character also separates hybrid specimens of Cx. pipiens x Cx. quinquefasciatus Say from Cx. restuans. The novel character was evaluated on gravid trap collected specimens from North Carolina, Pennsylvania, and Virginia and compared individually to polymerase chain reaction identification. Our study demonstrates that the novel character is highly accurate and more reliable when compared to two well-known character states published in the terminal couplets of common mosquito identification keys.

P-15 Identification of La Crosse, dengue, and chikungunya vectors collected from sticky traps using morphological and molecular methods

Brian Byrd, bdbyrd@wcu.edu, Marissa Taylor, Bruce Harrison, Monica Henry, Charlie Sither, Dawn Wesson, Manuel Amador, Roberto Barrera

Mosquito surveillance requires accurate, and often rapid, identification of medically important vectors. Rapid identification is especially important in urban surveillance programs where there is endemic disease or epidemic potential. Likewise, the introduction of exotic mosquito species is an increasing problem. Because container-inhabiting Aedes mosquitoes are poorly represented in CO2-baited light trap collections, the development of novel methods for the surveillance and control of these vectors remains a public health priority. Recently, low maintenance “sticky traps”, such as the Centers for Disease Control Autocidal Gravid Ovitrap (CDC-AGO), have proven useful for both surveillance and control efforts. A potential challenge with the use of these passive traps is the morphological identification of samples damaged by prolonged contact with the sticky surface or damaged during their removal from the trap. A recent field trial of the CDC-AGO in a La Crosse virus endemic area revealed that 10.3% of the sticky trap specimens could not be identified to species using standard identification keys for adult females. Here we report 1) useful “secondary” morphological characters for the identification of damaged container-inhabiting adult Aedes females in La Crosse virus endemic areas, and 2) a PCR protocol designed to identify Aedes mosquitoes collected by sticky traps used for La Crosse, Dengue, and Chikungunya surveillance. The combined use of both microscopic and molecular identification methods will allow investigators to identify damaged Ae. aegypti, Ae. albopictus, Ae. atropalpus, Ae. bahamensis, Ae. hendersoni, Ae. triseriatus, Ae. mediovittatus, and Ae. japonicus collected from passive sticky traps.

P-16 Population structure of *Anopheles nuneztovari* in Colombia

Nelson Naranjo-Diaz, dianitapaola@hotmail.com, Stefani A. Piedrahita, Yilmar Espinosa, Camilo Orozco, Juan C. Hernandez, Margarita M Correa

Anopheles nuneztovari is an important malaria vector with wide distribution in Colombia. The aim of this work was to evaluate genetic variability of An. nuneztovari through its distribution in Colombia using mitochondrial COI gene sequences. A COI parsimony haplotype network and spatial analysis of molecular variance (SAMOVA) independently detected three equivalent subclades, NW-W, NE and E Colombia that presented statistically significant, high genetic differentiation. Significant Mantel and partial Mantel tests showed that isolation by distance-IBD and isolation by resistance-IBR influenced this genetic differentiation. A signature of expansion was detected by the neutrality tests, that was further evidenced by COI Bayesian Skyline Plots, probably occurring at the end of the Last Glacial maximum (LGM). In conclusion, the COI analyses indicated that population differences of Colombian An. nuneztovari, as reflected by the three subclades, originated from a combination of demographic and ecological/geographic processes. Further studies should be directed to evaluate if these differences are influencing parasite transmission with the aim of designing targeted control strategies for An. nuneztovari in Colombian malaria endemic regions.

P-17 Effects of ascogregarine infections on container-inhabiting *Aedes* mosquitoes in southwestern Virginia

Joshua Bernick, joshua91@vt.edu, Sally Paulson

The cost of gregarine infection on the development of container-inhabiting Aedes mosquitoes from southwestern Virginia was assessed. Aedes albopictus, Aedes triseriatus, and Aedes japonicus are sympatric, share the same oviposition sites, and are all vectors for La Crosse virus (LACV). These container-inhabiting mosquitoes are also susceptible to infection from protozoan parasites in the genus Ascogregarina. Infection begins when mosquito larvae filter feed and gregarine oocysts are picked up from the aqueous environment. The gregarines continue their life cycle inside the host until the adult mosquito emerges. It is at this point that newly formed oocysts are released back into the water and the process starts over. Some oocysts can also remain in the adult mosquito and can be deposited at a new site, thus contributing to the spread and frequency of these protozoans. Ascogregarina infections in natural hosts are benign, however pathology can occur in cross-infections. Aedes albopictus and Ae. triseriatus are natural hosts of Ascogregarina taiwanensis and Ascogregarina barretti respectively. Although a gregarine has been found in Ae. japonicus in its normal range in Japan, it has not yet found in North American populations. We assessed the effects of parasite dose, cross-infection, and nutrition on survivorship, development rate, and adult size. Lastly, we measured the effect of gregarines on vector competence for LACV.

P-18 Dissecting the composition of the midgut microbiota of a Colombian field-collected malaria vector

Ana Priscila Bascuñan, pricebas@gmail.com, Yadira Galeano-Castañeda, Paula A. Urrea, Julián Rodríguez-Zabala, David Serre, Margarita M Correa

The attempts to improve strategies to reduce or eliminate malaria vector populations have dramatically increased due to emergence of insecticide-resistant Anopheles populations. Recently, it has been demonstrated that some bacteria of the mosquito midgut microbiota have anti-parasite activity, what suggests the importance of characterizing and evaluating mosquito midgut microbiota for alternative biocontrol agents. However, most studies have been performed in Asian and African vectors and little is known about the microbiota of the Latin-American

anopheline mosquitoes and its significance for parasite transmission inhibition. This study aims to characterize the composition of the midgut microbiota of a main Colombian malaria vector, Anopheles nuneztovari. Adult and mosquito larvae were collected in two Colombian malaria endemic regions. Specimens were identified by morphological keys and molecular methods. Samples were analyzed by culture-dependent and culture-independent techniques. Preliminary findings point at interesting bacterial diversity profiles between life stages and localities. Data are currently under final analysis by Illumina sequencing, which will supplement the identification of specific potential candidates for vector biocontrol.

P-19 Role of intestinal bacterial symbionts on growth and development of *Triatoma dimidiate*

Rene Montejo-Lopez, rene.montejo@espm.insp.mx, Teresa Lopez-Ordonez, Liliana Lopez-Tirado

Triatoma dimidiata is the main vector of Trypanosoma cruzi, in Central America and Southern Mexico. New control strategies include paratransgenesis: symbiotic bacteria are used to deliver molecules inside the insect. Bacteria naturally present in triatomine bugs are needed by the insect to grow and to complete development to adult stage. The objective in the present study was to assess the role of symbiotic bacteria on the development of T. dimidiata. Nine hundred eggs from a T. dimidiata laboratory colony were assigned to five groups: a control group (no treatment) and four groups which were treated to remove the microbiota present on them. Nymphs from the first group were fed on New Zeland rabbit blood; the second group (aposymbiont) was fed on rabbit blood but was kept in sterile conditions. Groups three, four and five were fed on blood supplemented with 1x105 CFU of Bacillus megaterium strain QM B1551, 1x105 CFU of Micrococcus luteus strain NCTC 2665, or with a combination of both bacteria, respectively. Eclosion, survival, mortality and bugs weight before and after blood meal were recorded. Survival was affected by treatments: 172 nymphs from the first group (control) reached 3rd. instar; none of the nymphs from the second group (aposymbont) survived to 3rd instar; 15, 6, and 23 nymphs from the groups three, four and five reached to 3rd instar respectively. Statistical analysis shows significate difference between groups survival (Pr(>|Chi|)<2.2e-16), but not between groups three and four (Pr(>|z|) 0.227817). These results strongly suggest that intestinal bacterial symbionts have a major role for growth and development in triatomines, but one or two bacteria species are not enough for the bugs to complete its development to adult stage.

P-20 Effect of malathion and bendiocarb selection pressure on a KDR *Aedes aegypti* field strain from Tapachula, Chiapas

Francisco Solis-Santoyo, fsosantoyo@hotmail.com, Alfredo Castillo-Vera, R. Patricia Penilla-Navarro, Juan Cisneros Hernandez, Alma Lopez-Solis, Americo D Rodriguez- Ramirez

Aedes aegypti is the main vector of dengue in Mexico and with the recently introduction of chikungunya virus in the country, vector control is even more needed, which is made mainly by the use of insecticides. Aedes aegypti populations are highly resistant to pyrethroids (PYR), which have been in use since more than a decade by the vector control program (VCP). In areas where high PYR resistance is present, the VCP recommends the use of organophosphates (OP) and carbamates (CAR). Given that during the application of an alternative insecticide resistance to the previously used is expected to decrease, we evaluated the selection pressure of malathion (OP) and bendiocarb (CAR) on a field resistant strain of Ae. aegypti. Selection pressure was exerted during seven generations by the CDC bioassay method and changes on the Val1016Ile and Phe1534Cys mutations frequencies were determined. Frequency of Ille 1016 decreased with the pressure of both insecticides while that of Cys 1504 increased with bendiocarb but remained

stable with malathion until the last generation exposed. This results suggest that resistance to PYR after malathion selection decreases, while it remains under the pressure with bendiocarb. These results are promising for prolonging the use of PYR.

P-21 Insecticide resistance status of *Aedes albopictus* from Tapachula, Chiapas, Mexico

Alma Lopez-Solis, adlopez@insp.mx, Francisco Solis-Santoyo, Patricia Penilla-Navarro, Alfredo Castillo-Vera, Juan Cisneros Hernandez, Americo Rodriguez Ramirez

Aedes albopictus has not been fully incriminated as a vector of dengue in Mexico, but as a secondary vector. However, with the introduction of the chikungunya virus in the country, the potential involvement of this vector acquires special relevance. Chemical control is still the major means for vector control and insecticide resistance may be a factor affecting control success. This species has been subject to selection pressure since its introduction, as ULV applications for Ae. aegypti have been taking place in areas where these two vectors coexists. Therefore, the susceptibility/resistance status of this species was determined by CDC bottles bioassays in two neighborhoods from Tapachula, Chiapas. Mortalities above 96% were recorded with the pyrethroids permethrin and deltamethrin in mosquitoes from both neighborhoods, 100% with the carbamates bendiocarb and propoxur, while with the organophosphates chlorpirphos and malathion, mortalities were 77% and 55% for the first neighborhood and 72% and 27% the second neighborhood. The low mortality observed for malathion, have been widely reported in Asia and USA. It could be possible that if Ae. albopictus came to Mexico for USA is presumed that the resistance being maintained by the use of malathion in growing areas of Tapachula

P-22 First molecular detection of *Rickettsia* sp. in *Rhipicephalus sanguineus* from Rocky Mountain spotted fever-endemic region of Northeastern Mexico

Aldo Ortega, agrortega@hotmail.com, Antonio Castillo, Sarai Cueto, Francisco Sánchez, Tere Valdes

Rickettsia species are the etiologic agent of Rocky Mountain spotted fever, an endemic disease in the northeastern Mexico, where it is transmitted by the bite of the brown dog tick Rhipicephalus sanguineus. This is the first record of molecular detection of R. rickettsii from brown dog ticks R. sanguineus collected in the northeastern Mexico. Objective: Identify the possible presence of Rickettsia species in internal organs of the brown dog tick using molecular tools. Methods: To determinate the possible presence of Ricketssia sp in brown dogs ticks R. sanguineus collected in domestic dogs, genomic DNA was extracted from 217 ticks (in 100 pools) collected in two Rocky Mountain spotted fever hyper endemic locations of Coahuila state in the Northeastern Mexico and subsequently tested for Rickettsia spp, Spotted fever group of Rickettsia, and R. rickettsii using quantitative PCR. Results: Rickettsia sp was detected in 2% (2/100) of pools of R. sanguineus. The presence of R. rickettsii in the macerated internal organs of ticks was determinate by PCR amplification of gltA genes derived by RpCS.877p and RpCS.1258n primers that amplifying in rank of 380-397 bp. Our study represent the first record of confirmed presence of Rickettsia sp in brown dog ticks Rhipicephalus sanguineus collected in domestic dogs of hyper endemic foci of Rocky Mountain spotted fever in the Northeastern Mexico. The presence of Ricketssia sp in ticks collected in dogs indicate the need of surveillance of ticks populations parasitized domestic dogs and the control measures for brown dog ticks by the public health officials.

P-23 Mosquito biodiversity and potential risk of chikungunya transmission in school environments in Southern, Mexico

Armando Elizondo-Quiroga, armando.elizondo@insp.mx, Samanta Lucía Del Río-Galván, Alejandro Gaitán-Burns, Maricela Laguna-Aguilar, Esteban Eduardo Díaz-González, Iliana Rosalía Malo-García, Ildefonso Fernández-Salas

Mosquitoes are the center of attention of researchers, entomologists, and public health workers because they transmit diseases causing millions of cases and deaths. The most important are dengue, chikungunya and malaria, but exist other dangerous arboviruses, i.e. West Nile fever, Eastern equine encephalitis, LaCross Encephalitis, etc. School environments are an important gathering point of children, most of them naive for these arboviruses. These environments are poor studied despite children spend much of their time there and the probability to be infected for any of this diseases increases. Knowing the vectors present in this kind of environments will allow to know the risk of transmission of determined arthropod-borne disease. The aim of this study was to know the mosquito biodiversity of school-environments in Chiapas, Mexico and their role in ecology in chikungunya transmission. The localities selected were Tapachula, Unión Juárez, Mazatán, Cacahoatán, Tuxtla Chico, Huehuetán, Huehuetán Estación, Tuzantán and Tuxtla Chico-Cacahoatán. The number of schools collected from these localities was 10, 2, 5, 14, 5, 6, 7, 6 and 7, respectively. A total of 1046 mosquitoes in 60 schools were collected by CDC backpack aspirator. The species collected were 596 Culex quinquefasciatus (57.0%), 370 Aedes aegypti (35.4%), 35 Aedes albopictus (3.3%), 19 Culex nigripalpus (1.8%) and 26 belonging to other 7 species (2.5%). Despite none of Ae. aegypti and Ae. albopictus were positive for chikungunya virus (CHIKV), school environments from Chiapas fulfill the ecological requirements for CHIKV transmission.

Education

P-24 Mosquito clip art

Molly Nee, mnee@mmcd.org

There is a need for high quality open sourced mosquito images for use in education and community outreach material. Many of the available non-copyrighted images are repeatedly used and reused for posters and presentations in the mosquito abatement world. Working for a government agency it is hard to justify spending money on licensing an image for use, so with my background in art I decided to create my own imagery. In creating posters and presentations I have produced a collection of images that I would like to share for free with those in the mosquito control community. Many of the images are life cycle diagrams and mosquitoes in different life stages. I will display the images on a web page in their original high quality file format, as well as a portable document format (pdf) so that they can be used or altered as the user sees fit.

P-25 Integration of mosquito ecology laboratory exercises into biological, geoscience, and environmental science courses: Using ovitraps to create authentic learning experiences

Brian Byrd, bdbyrd@wcu.edu, Diane Styers, Laura DeWald, Gideon Wasserberg

Discovery-based research and laboratory practices introduced early into educational experiences are associated with improved educational outcomes and retention in the sciences. Thus, there is renewed interest in exploratory educational exercises that can replace conventional laboratory studies and give students ownership of the research process and data. Here we describe the use of simple mosquito traps (i.e., ovitraps) to engage students and demonstrate basic and applied scientific principles. In these activities students are required to develop their own hypotheses, design their own experiments, collect data, and interpret the results. Over the course of more than

five years, we have incorporated the use of ovitraps in biology, geoscience, environmental science, and environmental health courses with both introductory and upper-level students. Our experiences using ovitraps in courses within the biological and health disciplines and community/citizen-science programs are presented.

Equipment

P-26 A comparison between two sampling methods for container-inhabiting *Aedes* in southwestern Virginia

Jake Bova, jbova86@vt.edu, Sally Paulson

Aedes triseriatus (Say), the eastern treehole mosquito and two invasive mosquitos, Aedes albopictus (Say) and Aedes japonicus (Theobald) are sympatric in southwestern Virginia. Aedes triseriatus is the primary vector of La Crosse virus (LACV) and the two exotic species are considered accessory vectors. The objective of this study was to compare the two sampling methods, ovitraps and gravid traps, to assess mosquito population density in forested areas. This study was conducted from June 2011 to August 2011 at two sites in the Jefferson National Forest and from late July 2015 to early September 2015 in the forest surrounding The Freshwater Mollusk Conservation Center at Virginia Tech. Twelve gravid traps and 24 ovitraps were used in 2011 and eight gravid traps and 16 ovitraps were used in 2015. Seed germination papers from ovitraps were collected each week, while gravid traps were set up and sampled during a 24-hour period twice each week. Adult mosquitoes from gravid traps and eggs on the seed germination papers were identified to species. During 2011 the relative abundance of Ae. triseriatus was significantly higher than that of Ae. japonicus and Ae. albopictus, whether measure by eggs in ovitraps or adults in gravid traps. The ratio of eggs collected by ovitrap to the number of adult mosquitoes sampled by gravid trap was 61.9. This ratio agrees closely with the fecundity of Ae. triseriatus, suggesting that either trap is effective for this species. For Ae. japonicus and Ae. albopictus, relative egg abundance collected via ovitrap was not comparable to adults collected via gravid trap. The low ratios suggest that ovitraps are not a sensitive method for collecting these 2 species. Data collected during 2015 are currently being analyzed.

Genetics

P-27 Genetic variability in *Aedes aegypti* (L.) (Diptera: Culicidae) in localities of north central region of Mexico

Rafael Perez, biol.rafal@gmail.com, Veronica Avila, Urbano Nava, Adriana Flores-Suarez, Aldo Ortega

There is currently a lot of molecular studies for Ae. aegypti either for identification, observe mutations, detect resistance genes. The study of genetic variation in a population of Ae. aegypti identifies subpopulations across geographic space. This study was conducted in the villages of north central Mexico, with the aim of determining the genetic variability of Ae. aegypti by uniparental mitochondrial ND4 gene in populations of northern central Mexico. 105 fourth instar larvae were collected extraction, amplification and DNA sequencing was performed, ND4 mitochondrial gene for genetic variability. The level of polymorphism found in the study populations was 0.39%, and the nucleotide diversity was worth 0.00625, in relation to the four haplotypes found high haplotype diversity of 0.750 was determined. The phylogenetic trees suggest the existence of four clades: Newest suggests to Torreon, Durango and Mapimí localities,

the oldest and best established corresponds to Camargo, Gomez Palacio and Lerdo San Pedro and two isolates that are Saltillo and Tlahualilo.

P-28 Phylogenetics of Aedini mosquitoes

John Soghigian, jsohjigian@clarku.edu, Marco Notarangelo, Todd Livdahl

For the majority of the twentieth century, mosquito genera were defined by morphological characters useful in differential identification. Since 2000, there has been a rapid increase in the number of genera within the family, primarily due to the elevation of subgenera from within the genus Aedes. These elevations were based upon cladistics analyses of morphological characters. More recently, additional analyses with the same characters called into question the elevation of these genera. Given the conflicting results of morphological analyses, the need for a molecular phylogeny of the medically important Aedes was apparent. Here we present the results from the most comprehensive molecular phylogeny of mosquitoes to date, with a particular focus on the genus Aedes, including more than seventy mosquito species. We used a database-driven approach in R to build a supermatrix from five gene regions for use in subsequent analysis with PhyML. We discuss our results, including implications for the monophyly of the genus Aedes.

Management

P-29 Challenges to the community health workers model for the control of malaria in Cameroon

Kathryn Mishkin, kemishkin@hotmail.com

The prevention and management of malaria is necessary to foster and achieve sustainable development. In 2013, over one million malaria infections were reported in Cameroon. In 2010, it was estimated that 40% of deaths in health centers were due to malaria, and that malaria caused 30% of hospitalizations, 50% of morbidity of children under five years old, and 45% of medical consultations. This paper analyzes Cameroon's National Malaria Control Program (NMCP) and its community-based malaria Community Health Worker (CHW) model in five regions across the country. Quality of service delivered by 157 CHWs providing malaria prevention education to roughly 18,800 people in the Southwest, Northwest, West, Adamawa, and the North regions are documented through key informant interviews provided by United States Peace Corps volunteers working with CHWs in five diverse sites in remote areas. Results from the volunteer interviews show that the CHW model is plagued by an insufficient number of CHWs for each work-space district, an inadequate system of providing quality education to the CHWs, a system that does not ensure that education be delivered in a culturally and religiously sensitive way, and a failing monitoring system. Recommendations are provided to strengthen the quality of service delivered by all implementing partners and CHWs including improved workforce development, increased access to education for the CHWs, incorporation of an engendered approach, and improved monitoring and evaluation competencies. The paper incorporates a sustainable development methodology to both reduce deaths caused by malaria and improve the domains of youth development, gender equity, and environmental protection.

New Product Trials

P-30 An experimental design to test the effectiveness of autodissemination stations containing pyriproxyfen in reducing juvenile *Aedes albopictus* populations

Isik Unlu, iunlu@mercercounty.org, Devi Suman, Yi Wang, Kim Klingler, Nicholas Indelicato, Gregory Williams, Scott Crans, Randy Gaugler

Aedes albopictus, the Asian tiger mosquito, is an aggressive, highly anthropophilic, day-biting mosquito with an expanding geographic range. This species continues to create a burden on existing mosquito control programs that are not adequately staffed or prepared to combat an urban container-inhabiting mosquito. The aim of this study was to reduce immature and adult Ae. albopictus populations by contamination of larval habitats using an autodissemination of the insect growth regulator pyriproxyfen. A previous study showed a clear decrease in the number of eggs collected from oviposition cups in the treatment sites; however, the data was inconclusive regarding adult population counts. Large variations in adult population numbers within the study sites and across sampling periods, in conjunction with restrictions on the number of sites that could be monitored, contributed to the inability to effectively isolate a treatment effect. Therefore, the study was redesigned to include more treatment and control sites and the number of mosquito traps at each site was doubled in order to provide some measure of replication. Barrier spraying with a residual adulticide was also included in the design to reduce inflation of adult mosquito numbers due to immigration from neighboring areas. A power analysis is included to explore the statistics attainable under the new design. Our poster will highlight the need and implementation of an experimental design necessary to gauge the efficacy of autodissemination studies.

P-31 New lethal ovitrap for control *of Aedes aegypti* and *Aedes albopictus*

Casey Parker, caseyparker@ufl.edu, Alexandra Chaskopoulou, Roberto Pereira, Philip Koehler

Aedes aegypti and Aedes albopictus are known to flourish in a variety of natural and residential habitats and are competent vectors of at least 22 different arboviruses including dengue and chikungunya. Their global distribution, anthrophilic nature, and vector competency make them species of interest for control. Lethal ovitraps with adulticiding activity have been used in recent years for the control of populations of these mosquitoes with encouraging results. A novel dual action lethal ovitrap (DDALO) with combined larviciding and adulticiding effects was designed to specifically target these mosquito species. The trap resulted in high adult mortalities during no-choice laboratory cage studies targeting gravid females and successfully prevented all deposited eggs from hatching. Aging of the DDALO maintained high adult mortalities and continued to prevent eggs from hatching. Choice bioassays that allowed multiple oviposition sites for gravid females resulted in a significant preference for DDALOs. Small cage population studies resulted in significantly lower populations of adult mosquitoes after 3 weeks in treated cages. Successful lab studies will be replicated in the field to determine the efficacy of DDALOs under natural conditions.

P-32 A smart Internet of Things (IoT) device for monitoring mosquito trap counts in the field while drinking coffee at your desk

Martin Geier, martin.geier@biogents.com, Michael Weber, Andreas Rose, Ulla Obermayr, Catherine Pruszynski, Michael Doyle

The BG-Counter is an autonomous new smart mosquito trap station that automatically differentiates mosquitoes from other insects, counts them, and wirelessly transmits results to a cloud server. Based on the industry-standard BG Sentinel, the BG-Counter enables real-time alerting as well as prediction models and historical analysis of infested areas. Vector control professionals can now establish surveillance programs with unprecedented data density, accuracy and real-time responsiveness, overcoming labor constraints associated with manual inspection. The BG-Counter can also sample local environmental data such as temperature, humidity, light, precipitation, or wind. Other sensor modules in the trap station's periphery supply additional data; for example, wireless standing water sensors provide "wet" or "dry" status

of nearby larval sites. As a result, both presence of adult mosquitoes and formation of breeding sites can be detected earlier and with greater precision, enabling faster, more targeted, and thus more effective, mosquito control. Since time-resolved mosquito abundance data are now routinely available, adulticiding can be performed when mosquitoes are the most active; also, the effectiveness of control measures can be validated immediately. The system is supported by a web-based database for storage of mosquito counts, geospatial and environmental data, and is automatically updated by the BG-Counters in the field. The data can be accessed, displayed and analyzed by the end user in a cloud-based "Management Central", and also exported to Excel at the push of a button. The intuitive graphical user interface can be accessed from PCs as well as smartphones and tablets.

Operations

P-33 Regular introduction of the invasive mosquito *Aedes albopictus* into the southern German state of Bavaria, via international traffic, between 2012 and 2015

Andreas Rose, andreas.rose@biogents.com, Ingeborg Schleip, Carola Kuhn, Egbert Tannich

The region south of the Alps, where the Ae. albopictus is well established, is also a popular vacation destination for Central Europeans. Transalpine highways connect the Mediterranean and Central Europe and often pass through Bavaria or its western neighbour, Baden-Württemberg. Starting in 2012, the German Federal Environment Agency began to support the surveillance for invasive mosquitoes in Germany. Surveillance in Bavaria was performed mainly in service areas on north bound highways, using ovitraps (OT) and BG-Sentinel (BGS) traps with BG-Lure. For each BGS, 3 OT were deployed. Prior to being emptied every fortnight, each BGS was additionally baited with CO2 for 24 h. The first Ae. albopictus adults in Bavaria were trapped in 2012. In 2013, one location was positive for adults, larvae and eggs, but an intensive inspection in spring 2014 gave no indication for overwintering. The largest numbers were recorded in 2015. Two locations were sampled every year until 2015; one (L1) with 3 BGS and 9 OT, the other (L2) with 4 BGS and 12 OT. In L1, one BGS was positive in 2012 (2♀♀), another BGS in 2013 (2♀♀) and in 2014 (1♀). The first OT was positive in 2013. In 2015, 3 BGS were positive at least once (total 7♀♀), 1 OT was also positive. In L2, the same 3 BGS were positive at least once in 2012 (3♀♀), 2013 (7♀♀), and 2015 (≥6♀♀). No BGS, but one OT was positive in 2014. In 2015, ≥4 OT were positive at least seven times. Summary: Ae. albopictus is regularly being introduced into Bavaria through international traffic from southern Europe, but no overwintering population was found yet. BGS traps were more sensitive in their detection than OT. This work was supported by the German Federal Ministry for Environment (BMU) through the Federal Environment Agency (UBA).

P-34 Establishing an *Aedes aegypti* threshold in Maricopa County, Arizona

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

The Maricopa County Environmental Services Department Vector Control Division mosquito surveillance program is targeted for West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV). We set 756 CDC CO2 traps weekly to detect the presence of two vectors, Culex quinquefasciatus and Culex tarsalis, and also capture Aedes aegypti in 12% of these traps. Chikungunya and Dengue fever have been identified at our southern border and residents often return diagnosed with one of these diseases. To prepare for future vector control activities, we need to design an adult surveillance system for this species. One method is to establish a local threshold for adult Ae. aegypti based on trap counts. In this study, we have compared data from the CDC CO2 traps with those such as BG-Sentinel, Fay Prince, and the Omni-Directional Fay

Prince. These traps are bulky, expensive and prone to theft and vandalism. We have sufficient data to correlate results from these traps with our CO2 traps. These are affordable and easily replaced if need be. Results from the field trials will be presented.

Other

P-35 Prevalence of head lice in lacandon children of the town of Lacanja Chansayab in Ocosingo, Chiapas

Maricela Laguna-Aguilar, maricela.laguna@insp.mx, Rosa María Sánchez Casas, Cuauhtémoc Villarreal Treviño, Illiana Rosalía Malo García, Nicole Achee, John Grieco, Ewry Arvid Zárate Nahón, Ildefonso Fernández Salas

Head lice is common in all social strata of Mexico although is difficult to establish its prevalence by the lack of epidemiological records. Despite the few studies about the topic is known to at least 13-60 % of the population in Mexico is infested. Indigenous groups are considered a vulnerable population for this reason a study was performed to determine prevalence of head lice in lacandon children. The community Lacanja Chansayab in Ocosingo, Chiapas, was visited during May and June for the study. A search for nits, nymphs or adults of head lice in children under 15 using a mechanical technique (lice comb) was performed. The results established a prevalence of 71.4% of children infected with at least one of the instar of the parasite. While 28.6 % of the children examined were negative to infestation. Positive children with Pediculus capitis 41.1 % were boys and 30.4% were girls. The scant attention and knowledge about this problem has allowed that the infestations of head lice continue out of control. So the vulnerable populations require renewed attention to the prevention and treatment of head lice

Public Relations

P-36 Interagency dengue prevention training in Martin County, Florida

Carlyn Porter, carlynp@martin.fl.us

The 2013 dengue outbreak in Martin County, Florida brought to light the importance of having a close working relationship between Martin County Mosquito Control and the Florida Department of Health. While our mosquito control program is managed through county government, our local health department is managed by the state. We must be actively engaged in bridging these two entities to improve our preparedness, both in practice and in public relations. In preparation for the 2015 mosquito season, Martin County Mosquito Control and the Florida Department of Health in Martin County conducted an interagency training exercise targeting the dengue vector Aedes aegypti in Rio, the epicenter of the prior outbreak. A joint press release concerning the exercise provided unified local news coverage and mutual promotion on television, radio, and newspapers, advising residents to also play their part in minimizing the domestic mosquito population. Entomological Inspectors were paired with Environmental Health Inspectors to cross-train in identifying mosquito breeding sites and larvae. Environmental Health Inspectors mapped out breeding locations using an ArcGIS-based application, while Medical Reserve Corps volunteers assisted in door-to-door resident outreach. Through combining resources and technologies, cross-training personnel, and improving communication between agencies, we were able to greatly increase the impact of our efforts. The result of this training exercise has been heightened public awareness, strengthened interagency relations, and an evident increase in inspection referrals from the health department, which has improved our surveillance capacity without increasing our budget or personnel.

P-37 Use of location-based social media for outreach and surveillance

Ada Barros Heiser, adab@placermosquito.org

Problem: Located on 1,400 square miles of diverse geography in Northern California, Placer County stretches for a hundred miles from the hot, dry, flat farmland and suburbs of south and west Placer through the the oak-covered foothills of Gold-Rush era towns to the craggy peaks, forests, tumbling rivers and clear alpine lakes of the Sierra Nevada mountains and Lake Tahoe. Along with Placer County's diverse landscape come a wide variety of mosquitoes and other vectors and issues related to those vectors. Conducting outreach to address those diverse vectors, issues, and human populations can be a big challenge for a smaller District. During certain times of year, there may be multiple vector issues in different geographic regions, and staff need to be able to conduct prevention education, as well as provide risk level information in a timely manner. Objective: The District needed to find a time and cost-efficient solution that could effectively provide specific geographical areas with appropriate prevention and risk-reduction messages. Methods: The District implemented social media outreach in 2010, and conducts ongoing evaluation of new and existing social media platforms that have the capability of "targeting" specific geographic areas with focused messages. The District currently holds Facebook, Twitter, Pinterest, Instagram, and Nextdoor social media accounts. Results: While Facebook allows advertising to selected criteria (gender, age, interests, location), Nextdoor provided map-based targeting of neighborhoods for no cost. We have found that Placer Nextdoor users are very civically engaged and are more likely to engage with the District. Although, like most social media, Nextdoor did not come without its own challenges.

Disease Vector II

108 Hourly distributions of EEEV vectors and respective infection rates in freshwater hardwood swamp habitats of southeastern Virginia

Jay Kiser, Jkiser@suffolkva.us, Karen Akaratovic, Charles Abadam

City of Suffolk Mosquito Control conducted a rotator surveillance trap study in southeastern Virginia from 2012 through 2015. Three sites were selected and trapped a total of three years on a weekly basis from May through September. At each site two rotator traps were set with each chamber representing one hour and covering a span of 4pm to 8am. During this study 28 mosquito species were collected with over 270,000 females in total. Of the species collected, Culiseta melanura (36%), Coquillettidia perturbans (21%), Culex salinarius (19%), and Culex erraticus (13%) were found with the highest frequencies. All four species had an average peak of activity during the hour of 9-10pm with a gradual decline throughout the later hours of the night. Cx. salinarius and Cx. erraticus had a smaller secondary peak during the hours of 5-6am and 6-7am respectively. During the 2013 and 2014 seasons, high rates of eastern equine encephalitis virus (EEEV) were found in the region's populations of Cs. melanura. At one of the rotator trap sites, where the highest frequency of EEEV occurred during these years, Cs. melanura was pooled by time and tested. Seventy-one EEEV positive Cs. melanura pools were found out of 752 pools tested. The hour containing the highest EEEV positive pools was 9-10pm, correlating with the hour of highest Cs. melanura activity and pools collected, but the hour with the highest EEEV minimum infection rate was during 4-5am. These findings help identify the most effective times for adulticide control efforts.

109 The potential role of invasive species in the transmission of La Crosse encephalitis viral genotypes associated with severe disease

Abelardo Moncayo, Abelardo.Moncayo@tn.gov, Katie Westby, Amy Lambert, Rebecca Trout-Fryxell

In July 2012, a 6-year-old boy from Union County, Tennessee, was seen at an emergency department after 2 focal seizures and symptoms consistent with viral encephalitis. Because of the geographic location, seasonal timing of illness, and clinical presentation, La Crosse encephalitis virus (LACV) was suspected as the cause of illness. His condition progressively deteriorated, and he was pronounced brain dead and died on day 5 after admission. At autopsy, an section of the brain was taken and sent to CDC for analysis. As part of this study we tested mosquitoes that we trapped near the child's home shortly after the child's post-mortem diagnosis. Field collected and laboratory-reared adults from egg collections were tested for LACV at the Tennessee Department of Health Vector-Borne Diseases Laboratory. Multisegment genomic sequence data were generated from the human and mosquito isolates by next-generation sequencing methods and confirmed these results by spot sequencing using traditional Sanger methods. Comparative sequence and phylogenetic analysis were also conducted. Our findings suggest that a limited range of LACV lineage I genotypes is associated with severe clinical outcomes. Although the mosquito species from which this strain was collected was Aedes triseriatus, two invasive species, Aedes albopictus and Aedes japonicus were found to be infected with LACV in field collections conducted in 2010. Maximum likelihood estimates (per 1,000 mosquitoes) were 2.72 for Ae. triseriatus, 3.01 for Ae. albopictus, and 0.63 for Ae. japonicus. The presence of an aggressive human biting vector, such as Ae. albopictus, is concerning as is presence of a 2nd invasive vector, Ae. japonicus, which may potentially expand the range of LACV.

110 First detection of St Louis encephalitis virus in California in 12 years

Gregory White, gbwhite@ufl.edu

Prior to the introduction of West Nile virus (WNV) in the Coachella Valley of California, St Louis Encephalitis virus (SLE) and Western Equine Encephalitis virus were commonly detected in routine mosquito and sentinel chicken surveillance. The year 2003, when WNV was first detected in Coachella Valley, marks the last time SLE was detected in the Coachella Valley and the rest of CA. In 2015, twelve years after SLE was last found in Coachella Valley, it was identified during routine mosquito surveillance. SLE in 2015 was initially detected in four mosquito pools in rural areas of the District. It was soon found in many more mosquito samples. During this same time period of SLE discovery, WNV was also detected within the District. The transmission patterns of SLE and WNV in the same area are described during this first year of SLE transmission in 12 years.

111 Disease surveillance in Harris County, Texas, 2015

Martin Reyna Nava, mreyna@hcphes.org

After last year's record-breaking number of West Nile virus (WNV) confirmed mosquito pools in Harris County, the presence of the virus in 2015 has been detected in only 355 mosquito pools up to date. This aligns with the activity detected in Harris County during the early years since the arrival of WNV to Texas in 2002. On the contrary, the number of WNV confirmed dead birds has reduced to a total of 12 highlighting the presence of the virus in avian host populations (mainly Blue Jays) to years in which the viral activity was very low in mosquito populations (2008 to 2011, and 2013). In addition, live bird sera has shown titer activity in the avian population for WNV, Saint Louis encephalitis (SLE), Western Equine encephalitis (WEE), and Eastern Equine

encephalitis (EEE) coinciding with the death of several horses in neighboring counties. Human cases amount to a total of 6 WNV, and 1 SLE confirmed cases with no related deaths.

112 Comparison of mosquito-based surveillance WNV indicators in an operational MAD setting

Roger Nasci, rnasci@nsmad.com, Christopher Xamplas

Efforts to predict risk of West Nile virus through mosquito-based surveillance have become common. Mosquitoes in communities across the United States are routinely collected, identified, counted, and tested for evidence of WNV through tests to detect WNV envelope proteins, RNA, or, rarely, live virus. Indicators of WNV risk are then derived from the resulting data. The WNV risk indicators most frequently used by state and local health agencies and mosquito abatement districts are: 1) Vector abundance, 2) Number of positive pools, 3) Percent of pools positive, 4) Infection rate, and 5) Vector index. In this presentation, we use 6 years of surveillance data collected in northern Cook County, Illinois to demonstrate the relationship among these indicators and their association with WNV risk, and discuss how these data are used to make operational decisions in the North Shore Mosquito Abatement District.

113 Spotlighting West Nile virus in Harris County: What a difference a year makes!

Cheryl Battle-Freeman, cbattle@hcphes.org, Yvonne Randle, Martin Reyna Nava, Monique Jackson, Joyce Landry, Mustapha Debboun

In Harris County, Texas, 2014 presented as an exceptional year regarding the total number of mosquito pools which were tested and confirmed positive for West Nile Virus (WNV). Mosquito samples were collected weekly, identified and pooled according to gender and species. After being processed, the pools were screened by the ELISA antigen capture test method, and positive samples were subsequently tested via the Rapid Analyte Measurement Platform (RAMP) for confirmation of test results. The total number of positive WNV mosquito pools (1,286) in 2014 was record breaking, far surpassing all other years since WNV was first discovered in Harris County in 2002. A comparative analysis between the total number of WNV confirmed mosquito pools in 2014, and the number obtained in 2013, shows a more than eight-fold increase. Additionally, in comparing data from 2014, as it pertains to the total number of confirmed positive WNV pools, to 2015, we find that 2014 continues to be highlighted as a stand-alone, remarkable year for WNV in Harris County, Texas.

114 Relevance of RT-qPCR cycle threshold values and antibody titers in free-ranging birds to the endemic/epidemic profile of West Nile virus transmission in Orange County, California

Tim Morgan, tmorgan@ocvcd.org, Carrie Fogarty, Robert Cummings, Kiet Nguyen, Laura Krueger, Albert Trinidad, Martine Jozan

The Orange County Mosquito and Vector Control District utilizes real-time RT-qPCR to detect West Nile virus (WNV) RNA and a blocking ELISA to determine WNV antibody seroprevalence in free-ranging birds. The PCR positive determinations are made for cycle threshold (Ct) values ≤ 30 using an envelope primer/probe assay. However, some avian specimens exhibit Ct values ranging from 30-40 throughout the year. It is unclear whether these values reflect a minimal viral load suggesting a recent infection, or a viral signature consistent with persistent infections. Attempts were made to recover live virus from all specimens with Ct values <40 by tissue culture isolation, and identification by neutralization and in situ ELISA. In addition, serological and PCR data from epidemic (outbreak) and endemic (non-outbreak) years from 2004

to 2014 were analyzed and compared. Monthly distribution of WNV seroconversions, antibody titers, and Ct values were analyzed for outbreak and non-outbreak years with emphasis on house finches (Haemorhous mexicanus) with recapture history. In outbreak years, we found significantly higher average antibody titers for the months of June, July, August, November, and December compared to those months in non-outbreak years. A better understanding of the antibody and PCR profiles of free-ranging avian hosts may help us refine our strategy for the surveillance of WNV transmission.

Disease Vector II

108 Hourly distributions of EEEV vectors and respective infection rates in freshwater hardwood swamp habitats of southeastern Virginia

Jay Kiser, jkiser@suffolkva.us, Karen Akaratovic, Charles Abadam

City of Suffolk Mosquito Control conducted a rotator surveillance trap study in southeastern Virginia from 2012 through 2015. Three sites were selected and trapped a total of three years on a weekly basis from May through September. At each site two rotator traps were set with each chamber representing one hour and covering a span of 4pm to 8am. During this study 28 mosquito species were collected with over 270,000 females in total. Of the species collected, *Culiseta melanura* (36%), *Coquillettidia perturbans* (21%), *Culex salinarius* (19%), and *Culex erraticus*(13%) were found with the highest frequencies. All four species had an average peak of activity during the hour of 9-10pm with a gradual decline throughout the later hours of the night. *Cx. salinarius* and *Cx. erraticus* had a smaller secondary peak during the hours of 5-6am and 6-7am respectively. During the 2013 and 2014 seasons, high rates of eastern equine encephalitis virus (EEEV) were found in the region's populations of *Cs. melanura*. At one of the rotator trap sites, where the highest frequency of EEEV occurred during these years, *Cs. melanura* was pooled by time and tested. Seventy-one EEEV positive *Cs. melanura* pools were found out of 752 pools tested. The hour containing the highest EEEV positive pools was 9-10pm, correlating with the hour of highest *Cs. melanura* activity and pools collected, but the hour with the highest EEEV minimum infection rate was during 4-5am. These findings help identify the most effective times for adulticide control efforts.

109 The potential role of invasive species in the transmission of La Crosse encephalitis viral genotype associated with severe disease

Abelardo Moncayo, abelardo.moncayo@tn.gov, Katie Wesby, Amy Lambert, Rebecca Trout-Fryxell

In July 2012, a 6-year-old boy from Union County, Tennessee, was seen at an emergency department after 2 focal seizures and symptoms consistent with viral encephalitis. Because of the geographic location, seasonal timing of illness, and clinical presentation, La Crosse encephalitis virus (LACV) was suspected as the cause of illness. His condition progressively deteriorated, and he was pronounced brain dead and died on day 5 after admission. At autopsy, an section of the brain was taken and sent to CDC for analysis. As part of this study we tested mosquitoes that we trapped near the child's home shortly after the child's post-mortem diagnosis. Field collected and laboratory-reared adults from egg collections were tested for LACV at the Tennessee Department of Health Vector-Borne Diseases Laboratory. Multisegment genomic sequence data were generated from the human and mosquito isolates by next-generation sequencing methods and confirmed these results by spot sequencing using traditional Sanger methods. Comparative sequence and phylogenetic analysis were also conducted. Our findings suggest that a limited range of LACV lineage I genotypes is associated with severe clinical outcomes. Although the

mosquito species from which this strain was collected was *Aedes triseriatus*, two invasive species, *Aedes albopictus* and*Aedes japonicus* were found to be infected with LACV in field collections conducted in 2010. Maximum likelihood estimates (per 1,000 mosquitoes) were 2.72 for *Ae. triseriatus*, 3.01 for *Ae. albopictus*, and 0.63 for*Ae. japonicus*. The presence of an aggressive human biting vector, such as *Ae. albopictus*, is concerning as is presence of a 2nd invasive vector, *Ae. japonicus*, which may potentially expand the range of LACV.

110 First detection of St. Louis encephalitis virus in California in 12 years

Gregory White, gwhite@cvmvcd.org

Prior to the introduction of West Nile virus (WNV) in the Coachella Valley of California, St Louis Encephalitis virus (SLE) and Western Equine Encephalitis virus were commonly detected in routine mosquito and sentinel chicken surveillance. The year 2003, when WNV was first detected in Coachella Valley, marks the last time SLE was detected in the Coachella Valley and the rest of CA. In 2015, twelve years after SLE was last found in Coachella Valley, it was identified during routine mosquito surveillance. SLE in 2015 was initially detected in four mosquito pools in rural areas of the District. It was soon found in many more mosquito samples. During this same time period of SLE discovery, WNV was also detected within the District. The transmission patterns of SLE and WNV in the same area are described during this first year of SLE transmission in 12 years.

111 Disease surveillance in Harris County, Texas, 2015

Martin Reyna Nava, mreyna@hcphes.org

After last year's record-breaking number of West Nile virus (WNV) confirmed mosquito pools in Harris County, the presence of the virus in 2015 has been detected in only 355 mosquito pools up to date. This aligns with the activity detected in Harris County during the early years since the arrival of WNV to Texas in 2002. On the contrary, the number of WNV confirmed dead birds has reduced to a total of 12 highlighting the presence of the virus in avian host populations (mainly Blue Jays) to years in which the viral activity was very low in mosquito populations (2008 to 2011, and 2013). In addition, live bird sera has shown titer activity in the avian population for WNV, Saint Louis encephalitis (SLE), Western Equine encephalitis (WEE), and Eastern Equine encephalitis (EEE) coinciding with the death of several horses in neighboring counties. Human cases amount to a total of 6 WNV, and 1 SLE confirmed cases with no related deaths.

112 Comparison of mosquito-based surveillance WNV indicators in an operational MAD setting

Roger Nasci, rnasci@nsmad.com, Christopher Xamplas

Efforts to predict risk of West Nile virus through mosquito-based surveillance have become common. Mosquitoes in communities across the United States are routinely collected, identified, counted, and tested for evidence of WNV through tests to detect WNV envelope proteins, RNA, or, rarely, live virus. Indicators of WNV risk are then derived from the resulting data. The WNV risk indicators most frequently used by state and local health agencies and mosquito abatement districts are: 1) Vector abundance, 2) Number of positive pools, 3) Percent of pools positive, 4) Infection rate, and 5) Vector index. In this presentation, we use 6 years of surveillance data collected in northern Cook County, Illinois to demonstrate the relationship among these indicators and their association with WNV risk, and discuss how these data are used to make operational decisions in the North Shore Mosquito Abatement District.

113 Spotlighting West Nile virus in Harris County: What a difference a year makes!

Cheryl Battle-Freeman, cbattle@hcphes.org, Yvonne Randle, Martin Reyna Nava, Monique Jackson, Joyce Landry, Mustapha Debboun

In Harris County, Texas, 2014 presented as an exceptional year regarding the total number of mosquito pools which were tested and confirmed positive for West Nile Virus (WNV). Mosquito samples were collected weekly, identified and pooled according to gender and species. After being processed, the pools were screened by the ELISA antigen capture test method, and positive samples were subsequently tested via the Rapid Analyte Measurement Platform (RAMP) for confirmation of test results. The total number of positive WNV mosquito pools (1,286) in 2014 was record breaking, far surpassing all other years since WNV was first discovered in Harris County in 2002. A comparative analysis between the total number of WNV confirmed mosquito pools in 2014, and the number obtained in 2013, shows a more than eight-fold increase. Additionally, in comparing data from 2014, as it pertains to the total number of confirmed positive WNV pools, to 2015, we find that 2014 continues to be highlighted as a stand-alone, remarkable year for WNV in Harris County, Texas.

114 Revelance of RT- qPCR cycle threshold values and antibody titers in free-ranging birds to the endemic/epidemic profile of West Nile virus transmission in Orange County, California

Tim Morgan, tmorgan@ocvcd.org, Carrie Fogarty, Robert Cummings, Kiet Nguyen, Laura Krueger, Albert Trinidad, Martine Jozan

The Orange County Mosquito and Vector Control District utilizes real-time RT-qPCR to detect West Nile virus (WNV) RNA and a blocking ELISA to determine WNV antibody seroprevalence in free-ranging birds. The PCR positive determinations are made for cycle threshold (Ct) values ≤ 30 using an envelope primer/probe assay. However, some avian specimens exhibit Ct values ranging from 30-40 throughout the year. It is unclear whether these values reflect a minimal viral load suggesting a recent infection, or a viral signature consistent with persistent infections. Attempts were made to recover live virus from all specimens with Ct values <40 by tissue culture isolation, and identification by neutralization and in situ ELISA. In addition, serological and PCR data from epidemic (outbreak) and endemic (non-outbreak) years from 2004 to 2014 were analyzed and compared. Monthly distribution of WNV seroconversions, antibody titers, and Ct values were analyzed for outbreak and non-outbreak years with emphasis on house finches (*Haemorhous mexicanus*) with recapture history. In outbreak years, we found significantly higher average antibody titers for the months of June, July, August, November, and December compared to those months in non-outbreak years. A better understanding of the antibody and PCR profiles of free-ranging avian hosts may help us refine our strategy for the surveillance of WNV transmission.

213 The Invasive Mosquto Project (IMP): A monitoring and educational tool

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

The Invasive Mosquito Project is a crowdsourced mosquito monitoring network aimed at identifying the distribution of invasive mosquito species. The network consists of high school teachers collecting container breeding mosquitoes using standardized oviposition collection and mosquito rearing protocols as a part of their biology lesson plans for school curriculum. The lesson educates students about mosquitoes, vector-borne diseases, hazards, and personal protection, while simultaneously collecting valuable mosquito species distribution data. To aid in recruitment of collaborators, teachers are provided written lesson plans, power point

presentations, and written protocols. The mosquito data (abundance and distribution) is uploaded to a central website (www.citizenscience.us). Mosquito eggs and voucher specimen adults are mailed to a central USDA repository or to local AMCA members, where identifications will be confirmed and specimens preserved. This presentation is to introduce and alert AMCA members that teachers may be contacting them for help with mosquito identification for this project.

Remotely Piloted Aircraft Symposium

115 Development of unmanned aerial systems for mosquito control- Part I

Randy Gaugler, gaugler@rci.rutgers.edu, Greg Williams, Scott Crans, Shuan Kenny, Ishik Unlu, Yi Wang

Unmanned aerial systems (UAS) offer enormous operational potential, but only if specific applications can be successfully delegated to them. A series of entirely autonomous field trials have been conducted with larvicide and adulticide ULV modules mounted on a heavy-lift hexacopter including take off, sprayer activation/deactivation, and return to launch on a multi-waypoint spray mission. Rotor wash dynamics were investigated by fixing smoke bombs to the UAS, enabling visualization of the unique air vortices forming around a multiple rotor configuration. These vortices have particularly important implications for ULV sprayers. Our waterproof quadcopter has demonstrated the ability to locate and land in small water pools to provide real-time video surveillance of larvae. The addition of a 3D printer has accelerated module development, including printing of a next generation larval collector.

116 Development of unmanned aerial systems for mosquito control- Part II

Gregory Williams, gwilliams@hudsonregionalhealth.org, Randy Gaugler, Scott Crans, Shaun Kenny, Isik Unlu

In Part II we continue our discussion on UAS for mosquito control. In response to the limited efficacy of liquid larvicides in salt marsh environments discussed previously, dispensing units have been developed for solid larvicides. We demonstrate the accuracy and precision of a module for dropping larvicide briquettes as well as a granular applicator. We also present details of a new large octocopter that was constructed to improve flight time and payload capacity. Given the limitations of satellite imagery, we present the potential of a fixed-wing UAS to create up to date, high resolution, multispectral maps useful for analysis of potential mosquito habitat.

117 UAS operational surveillance technology

Bill Reynolds, breynolds@leateam.com

Remote Piloted Aircraft (RPA) or unmanned aerial systems (UAS) are rapidly changing the productivity and time consuming tasks associated with operational mosquito surveillance and aerial applications. Developed specifically for mosquito control operations, multi-spectral imagery inclusive of geo-referenced mosaic images provide the ability to easily identify potential breeding sites, create flight plans through mission planning software and guide the RPA to land on the water. Additionally, real time high resolution video imaging assists in determine if mosquito larvae are present. The same RPA provides the user the ability to apply larvacide(s) while onsite if warranted. If larval populations are abundant, flight planning missions can be developed and uploaded to larger RPA's that are capable of larvaciding up to one acre per minute. These same RPA's are also capable of adulticiding up to thirty acres per minute

118 Commercial aerial larviciding applications – Have we made it?

Bill Reynolds, breynolds@leateam.com

Several commercial aerial applications of liquid and granule larvacides were performed in 2015 using Remote Piloted Aircraft (RPA). This presentation will provide a brief historical perspective of all the Federal and State challenges and requirements that resulted this game changing technology, now available in the United States.

119 Using multi-spectral imagery paired with infrared technology to identify mosquito breeding habitat in the Florida Keys

Heidi Murray, hmurray@keysmosquito.org

The Florida Keys Mosquito Control District tested a high resolution multi-spectral camera paired with infrared technology to enhance detection and treatment of mosquito breeding habitat. We conducted a field trial to ascertain the feasibility of determining where mosquito breeding water is located using airborne imagery. In August 2015, a multi-spectral camera was paired with infrared technology and flown over a variety of mosquito breeding habitat in the Florida Keys. Sites ranged from 0 – 100% cover and field inspectors ground-truthed these areas during the test flights for comparison. Distance of the aircraft from the ground, aircraft speed, camera aperture and shutter speed are all important factors in determining effectiveness. Early insights show a potential for adapting a camera to our specific needs. It remains to be seen whether these flights will be cost effective. Results from this study will likely influence whether airborne imagery can be used to enhance mosquito control operations in our district.

120 Use of remotely piloted aerial vehicles for chemical delivery in pest control

Ken Giles, dkgiles@ucdavis.edu

The use of Remotely Piloted Vehicles (RPV's) for aerial delivery of pest control agents involves additional technical and regulatory considerations beyond their use for surveillance, imagery and asset tracking. Payload capacity, aircraft flight endurance and power for payload handling and dispersion are technical issues related to the aircraft. The effects of aircraft wake turbulence on the dispersion of released solids and liquid droplets and the subsequent deposition and drift are well known for manned aircraft yet relatively undocumented for RPV's. Regulatory issues include the definition of operator and pilot certifications, the label compliance related to RPV aerial application and worker exposure to bioactive agents in payload. This presentation will present results from three years of work in the field spray environment with a 100 kg remotely piloted helicopter delivering pesticides in an agricultural environment and the outlook for RPV deployment in vector control, invasive species and other pesticide applications.

121 Dropping larvicide from 20 feet: A UAV evaluation

Jennifer Henke, jhenke@cvmvcd.org

The Coachella Valley Mosquito Control District examined the efficacy of a larvicide application by an Unmanned Aerial Vehicle (UAV) with Leading Edge Associates. The aircraft was piloted by Leading Edge Associates and fitted to make a liquid application of VectoBac WDG and VectoLex WDG (1:3 mixture) to a duck club pond 200 feet by 1300 feet. Ten floating cages were placed throughout the pond, each with 25 third-instar *Culex tarsalis*. After 6 hours, four of the cages had 100% larval mortality, and one cage had a hole which may have allowed larvae to escape. After 24 hours, two larvae were alive in the remaining five cages. Larvae in control cages in an untreated pond had 4.5% mortality at 24 hours. Volume density was also measured for the

application. Average volume density was 0.98 gallons per acre with a range of 0.04 to 2.83 on the cards that remained upright during the application.

Larval Control I

122 The evaluation of VectoPrime FG in a microbial bucket and field trip, including the aerial calibration of VectoPrime FG and its operational use in Pinellas County

Leanne Lake, llake@pinellascounty.org

The goals of the microbial bucket and field trial of VectoPrime FG was to prove or disprove its effectiveness on asynchronous breeding of multiple species; to prove or disprove if effectiveness on all instars of larvae including third and forth instars; and to understand the new dual mode of action of VectoPrime FG. We first conducted two separate microbial bucket trials; the first using all instars of *Culex quinquefasciatus* and the second using all instars of *Aedes taeniorhynchus*. Using field collected larvae and rabbit food infused water, we observed how the product works on each species and instars at a rate of 5 lbs/acre. After learning how the dual mode of action functions and what to expect, we calibrated our helicopter to a rate 4.5 lbs/acre for a field application. We chose a treatment site that was actively breeding two different species at all instars. Using a specific protocol, we set up 18 dip locations, collection totes, and rain gauges for data collection. We then applied the product aerially and tracked all valuable data. In conclusion, we found that VectoPrime FG effectively controlled all stages of multiple species on the treatment site at a rate of 4.3 lbs/acre.

123 Physiochemcial properties of pyriproxyfen as Aedes mosquitoes?

Kamal Chauhan, kamal.chauhan@ars.usda.gov

The environmental impact of pesticides demands a need for biopesticides or sustainable toxicants that can substitute for existing synthetic chemicals. Our research is aimed at developing ecologically friendly insect toxicants from renewable resources.**Pyriproxyfen** is a pyridine-based pesticide that has been found to be effective against a variety of arthropods. Pyriproxyfen is being successfully explored as a choice larvicide against container or stagnant water breeding *Aedes aegypti* (red eye) and *Ae albopictus*(the Asian tiger mosquito) mosquitoes. These are the most invasive and aggressive disease vectors in subtropical regions of the world. High potency (measured in ppb) and short half life period make pyriproxyfen one of the most desirable larvicides for mosquito control and there is continued interest in its potent chemistry. Pyriproxyfen is stable to hydrolysis and its water solubility is 0.367 ppm. The presence of increased organic matter in stagnant water increases half life (3.6 days to 25 days) and causes pyriproxyfen to become more persistent compromising its dispersion. Our approach for modulating physiochemical properties of pyriproxyfen thus focused on reducing lipophilicity and increasing bioavailability in aqueous media.
In our laboratory and through collaborative research we are modulating pyriproxyfen\analogues for reduced lipophillic character without compromising binding of pyriproxyfen to JH site receptors and larvicidal potency. We have successfully developed novel larvicide candidates with desired properties. The process included screening through designing, in-silico docking, synthesis, in-vitro bioassay and ultimately evaluating mosquito larvicidal potency

124 Does pyriproxyfen prevent pupation or adult eclosion of Aedes mosquitoes?

Kristen Stevens, kcstevens93@ufl.edu, Philip Koehler, Roberto Pereira

Effects of pyriproxyfen on *Aedes aegypti* and *Aedes albopictus* larvae were evaluated. Using 120-ml cups filled with 50-ml of 1, 3 and 10 PPB concentrations of pyriproxyfen in water, observations were made every 24 hours. Results showed that higher doses delay mosquito development and kill larvae, whereas lower doses did not delay development but killed mosquito pupae. Pyriproxyfen effectively controlled mosquito larvae, preventing pupation at high does and adult eclosion at lower doses.

125 The efficacy of Natular™ G30 and MetaLarv® S-PT in Lee County, Florida during summer of 2015

Rachael Morreale, morreale@lcmcd.org, T. Wayne Gale, Jonathan Hornby

Natular™ G30 and MetaLarv® S-PT were applied at various locations throughout Lee County, FL prior to flooding as a prehatch application. Natular™ G30 was applied at a rate of 8 lbs/ac and MetaLarv® S-PT was applied at 4 lbs/ac. Initial product applications were determined based on applications made the previous year. A subsequent application was made once the initial products began to fail. Treated areas were inspected regularly to monitor larval mosquito populations in order to evaluate the duration that the product remained effective. Larval presence inspection consisted of three replicates of three dips throughout each treatment area as water was available. The cost effectiveness of the applications were determined by the number of larval broods prevented due to the controlled release of the products compared to single brood larvicide applications and the associated costs.

126 Evaluation of Natular XRT for larval control in a vacuum sewer system in York County, Virginia

Leah Henretta, leah.aguilar@yorkcounty.gov, Elizabeth Hodson, Michelle Slosser, Derek Drews

York County, VA utilizes septic, gravity, and vacuum sewer systems with an ongoing goal of converting all septic users to gravity or vacuum. Vacuum pots, a component of the vacuum system, are a mosquito breeding source; however they have never before been analyzed or treated in York County. The purpose of this study is to establish baseline data on the mosquito species utilizing the pots and to observe the effectiveness of Natular XRT (Spinosad 6.25% AI) for larval control in the pots. There are currently 2718 vacuum pots in use in the County with plans to double the number within 7 years. Staffing levels and costs have necessitated that the larvicide used in the pots provide season-long control (25 wks) with a single treatment. Forty-four vacuum pots were randomly chosen in the Dare area of York County; 24 pots were treated with one Natular XRT tablet and the other 20 were untreated. Treatments were applied in May, 2015 and pots were sampled biweekly for larvae using a standard dipper for 180 days. The Natular treated pots had significantly fewer *Culex* and *Aedes* larvae than the control pots throughout the study. Factors that influenced the attractiveness of vacuum pots as breeding sites included location, and the amounts of water and organic matter present. Vacuum sewer systems are used in many states and are likely to become more commonly used due to their lower cost, environmental impact, and energy requirements in comparison to alternate systems. Conversion from septic to vacuum sewer provides nutrient reduction credits to the locality, a requirement of the Chesapeake Bay TMDL plan. Our findings suggest that the use of Natular XRT in vacuum pots is an effective control of mosquito larvae for up to 180 days.

128 First report of *Bacillus sphaericus* resistance in wild populations of *Culex pipiens* (Diptera: Culicidae) in California with note on susceptibility to other pesticides

Tianyun Su, tsu@wvmvcd.org, Min-Lee Cheng, Jennifer Thieme, Matt Ball, Chris Ocegueda

Bacillus sphaericus Neide, recently renamed as *Lysinibacillus sphaericus* Meyer and Neide, is a spore-forming bacterium that possesses various levels of larvicidal activity against some mosquito species. Products based on most active strains such as 2362, 2297, 1593, C3-41 that bear binary toxins have been developed to combat mosquito larvae worldwide. Resistance in wild *Culex* mosquito populations has been reported since 1995 from France, Brazil, India, China and Tunisia. Laboratory studies to evaluate resistance development risk have been conducted by many groups of scientists. Management tactics to prevent resistance development and restoration of susceptibility to *B. sphaericus* have also been developed and implemented. Product based on *B. sphaericus* strain 2362 was registered in California in 1996, and its use for mosquito control has increased considerably since invasion of West Nile virus. This report documents the first occurrence of high levels resistance to *B. sphaericus* in a natural population of *Cx. pipiens* in northern California, USA, where resistance ratio was 537.0 at LC_{50} and 9,048.5 at LC_{90} as compared with susceptible laboratory colony. Susceptibility profile to other groups of pesticide in this field collection was also established. Resistance management and susceptibility monitoring strategies are discussed

129 Cross resistance to *Lysinibacillus sphaericus* in spinosad-resistant *Culex quinquefasciatus* is overcome by a Cyt1A-BinA recombinant of *Bacillus thuringiensis*

Tianyun Su, tsu@wvmvcd.org, Min-Lee Cheng, Margaret Wirth, Jennifer Thieme, Hyun-Woo Park, Denis Bideshi, Brian Federici

Spinosad, a biopesticide consisting of spinosyn A and D, is produced by a naturally occurring, soil-dwelling bacterium, *Saccharopolyspora spinosa* Mertz and Yao. As a new class of polyketide-macrolide insecticides, spinosad has pesticidal activity after ingestion and cuticle absorption against a broad spectrum of susceptible insect species, acting at the nicotinic acetylcholine (nAChR) and γ-aminobutyric acid receptors, causing rapid excitation of the insect nervous system. High resistance to spinosad occurred in response to laboratory selection in *Culex quiquefasciatus* (Su and Cheng 2014a). The resistance ratio after 70 generations of selection reached 52,330-66,447 fold at LC_{50} and 2,934,136-3,522,789 fold at LC_{90} as compared with susceptible reference population. Cross resistance to *Lysinibacillus sphaericus* (VectoLex and Spheratax) evolved simultaneously during development of resistance to spinosad. The cross resistance reached 81-125 fold at LC_{50} and 9,471-20,744 fold at LC_{90} during generations F_{29-37} (Su and Cheng 2014b). At generation F_{68}, this spinosad-resistant population showed 9.5-34.3 fold cross resistance to *L. sphaericus* 2362 spore/crystal mixtures, but only 0.9-1.2 fold cross resistance to a recombinant strain of *B. thuringiensis* (Bt) engineered to produce a chimeric protein consisting of Cyt1Aa fused to BinA, the latter being the toxic moiety of the *L. sphaericus* binary mosquitocidal protein. The significance of the recombinant Bt Cyt1A-BinA fusion protein in spinosad resistance management in mosquitoes will be discussed.

Operations and Management

130 A cost-benefit analysis of illustrative *Aedes albopictus* eradication and management plans in Brisbane, Queensland

Jonathan Darbro, Jonathan.Darbro@qimr.edu.au, Paul Mwebaze, Yara Halasa, Brian Montgomery, Donald Shepard, Greg Devine

Aedes albopictus is one of the most invasive organisms on the planet, is currently present in the Torres Islands just north of mainland Australia, and has been intercepted in mainland Australia over the last several years, yet there is no response plan in place to combat an infestation in a

residential or commercial zone should one occur. We estimate cost-benefit analyses for 1) an illustrative response plan to eradicate a hypothetical infestation of *Ae. albopictus* in Brisbane, QLD, and 2) a sustained management plan if the mosquito becomes established. The eradication plan is based on establishing an 'eradication zone' (EZ) with a radius of 500m, carrying out larviciding activities in yards within the EZ and harborage spraying where appropriate, as well as carrying out surveillance inside and outside the EZ. The sustained control scenario contains elements of nuisance mosquito control but is mostly a vector control response to cases of dengue or chikungunya, modeled on public health activities in Cairns, where dengue outbreaks occur annually. The benefits of *Ae. albopictus* eradication or management are based on a willingness-to-pay survey of residents in Merced and Monmouth Counties, New Jersey, with adjustments made for inflation, currency differences and Australian GDP. Sensitivity analysis indicates that variables associated with the most uncertainty are estimates of perceived benefits, suggesting that willingness-to-pay surveys specific to Southeast Queensland are warranted. Despite differences between New Jersey and Queensland residents with regards to perceptions and expectations relating to mosquitoes and mosquito control, the cost-benefit ratio is quite favorable. Implications and future directions will be discussed.

131 Moving rapidly from discovery to commercial collaboration: How to prepare your data to accelerate new mosquitocide delivery

Rajeev Vaidyanathan, rvaidyanathan@clarke.com

University and government labs commonly screen derivatives from plants, bacteria, fungi, and animals to identify new molecules that kill larvae and adults of mosquitoes. The strength of research labs is their ability to screen thousands of molecules, conduct quantitative structure-activity relationship (QSAR) analysis on bioactive hits, design/synthesize/evaluate analogs, and even determine modes of action (MOA). After accomplishing these tasks, research labs might seek an industry partner with expertise in formulation chemistry, toxicology, environmental impact studies, new product registration, manufacturing, and commercialization. Our goal is to define what specific experiments, datasets, and characterizations can ensure the rapid transition of primary discovery work to formulation and new product development. We will draw examples from real projects to discuss technology transfer, writing a statement of work (SOW), experimental design, types of bioassays, EC_{50} calculations, and concentration guidelines/cutoffs. We will also discuss considerations of chemistry, such as temperature and photostability, hydrophobicity and solvent acceptability, products of degradation, and batch consistency in the case of botanical derivatives or recombinant products.

132 Mosquito control in an area of massive infestation of Aedes albopictus in South-West Germany

Nobert Becker, norbertfbecker@web.de, Björn Pluskota, Stefanie Schön, Egbert Tannich, Jonas Schmidt-Chanasit, Carola Kuhn, Artur Jöst

Aedes albopictus is widely distributed in Southern Europe and is occasionally introduced by vehicle transport into Central European countries. Accordingly, in 2005 the German Mosquito Control Association (KABS) started a large-scale monitoring program along the highway A5, an expected port of entry for mosquitoes from Italy and France, resulting in the first record of *Ae. albopictus* in Germany in 2007. In 2012, a national-wide surveillance programme has been initiated by the German Government with special emphasis on motorways connecting Germany with Southern Europe. Results from ovitraps and CO_2-/odour-baited traps at resting stations indicated an enormous increase in numbers of introduced *Aedes albopictus* females between 2007 and 2015. Moreover, in 2014 *Ae. albopictus* was detected in a garden area in the city of Freiburg

in Southern Germany, close to a railway station where hundreds of trucks arrive every day on trains from Italy. In 2015, an intensive monitoring program has been carried out in and around this garden area. The results indicate a massive infestation with *Ae. albopictus* as proven by a CI of 28%, and trapping of large numbers of up to 35 imagines per BG/GAT-trap/night. A control program was immediately implemented including circulating of information for the gardeners and the general public to eliminate breeding sites. The garden plots and residential areas around the garden area were inspected and positive containers were eliminated or treated with Bti. *Ae. albopictus* adults were screened for viruses with molecular techniques to assess the risk for virus transmission. Until now, all mosquitoes were tested negative for flaviviruses and alphaviruses. The success of the operation is continuously evaluated.

133 Realizing the benefits of Esri AcrGIS online and cloud technology for mosquito control operations

Ryan Pierson, ryan@elecdata.com

Many mosquito control organizations are missing the best way to share and use maps and may not be aware of the Esri ArcGIS Online Entitlement that is included with current ArcGIS licensing. This presentation will discuss and demonstrate the functionality available in the ArcGIS Online environment that directly benefits mosquito control organizations and the communities that they serve. The ArcGIS Online cloud-based service provides many advanced GIS analysis, web mapping, and data integration tools that can streamline traditional mosquito control operations and allow rapid access to information that aids decision making. This presentation will demonstrate several ArcGIS Online tools that will directly benefit mosquito control organizations including an operations dashboard, web map creation, public engagement, and data integration. To conclude the presentation, we will show the latest enhancements of our Sentinel GIS and FieldSeeker GIS software.

134 Controlling mosquitoes on private property: Balancing statutory authority with landowners' preference

Douglas Carlson, doug.carlson@irmosquito2.org, Diane Richards

For more than a half century, most mosquito control programs in Florida have faced the challenge of carrying out their control activities on property which they do not own. In the 1950s and 1960s, most property owners were very willing to allow programs to do whatever was necessary to control mosquitoes produced on their property. As mosquito populations came under better control, and as newer owners to the area are not fully aware of the problems that can emanate from their property if left unabated, some property owners now want to have a greater say in what control measures are conducted on their land. This presentation will review some examples of this potential conflict between mosquito control and landowners and explain how in some instances compromises have been achieved.

135 Field Data Submission (FDS) – management system of biosurveillance data and vector surveillance management

Luke Mitchell, lukemitchell315@gmail.com, Yvonne-Marie Linton

Specimen data (geo-references, locality and habitat variables) are critical to modern biosurveillance programs, allowing development of spatially and temporally informed vector borne disease risk assessment**.** Collecting, handling, compiling, formatting and the presentation of vector specific data is time-consuming and prone to errors. The continued assimilation of information, including morphological and molecular identification for further utility in

biosurveillance tools (VectorMap, BOLD etc) requires data to be standardized, logical and malleable to allow compatibility, integrity and usability. There is also an inherent need to simplify and update the procedures for the collection of critical biosurveillance data in the field, taking into consideration environmental factors, physical restrictions (weight, usability, training burden, cost) and changing data requirements.

In the 21st Century, data assimilation can begin on hand-held devices (cell phones, tablets) and be automatically compiled, formatted, saved and shared, reducing time and effort while increasing data granularity and usefulness. To this end, WRBU have developed a cost effective and simple data management system, to aid in all aspects of vector based activity and data management, which is easily adaptable for other users' project specific outcomes, while assisting with adding conformity to the vector control industry.

214 Computer Methods: Test trial results

David DeMay, ddemay@keysmosquito.org

Last year a presentation entitled "Computer Methods to Maximize Larviciding" identified three methods namely: a.) indicator plant html color codes (via GIMP), b.) Monroe County Habitat maps (GIS), and c.) elevation contour maps (sketchup.com) were used to identify optimal mosquito breeding sites in the Florida Keys. A dozen entomological inspectors were selected and assigned ten or more optimal mosquito breeding sites to monitor. The initial site visit involved determination of GPS coordinates, photography, assessment of potential for mosquito breeding, and determination of relative indicator plant coverage of the site. Reasonably convenient sites (10 per inspector) are monitored weekly through the 2015 wet season (June to November). Data for these sites are tabulated and statistically compared to sites independently chosen by the inspectors to determine the efficacy of the computer methods.

137 Sustainability: Measuring the impact on mosquito control operations

Mark Smith, mmcd_mes@mmcd.org

The Metropolitan Mosquito Control District (MMCD) strives to implement sustainability practices in all aspects of our operations. Organizations should understand and analyze the time, effort and costs of handling byproducts of their operations. MMCD is working as a partner with our vendors to reduce packaging waste and improve our overall efficiency. Our employees are continuing to find new ways to reduce our impact on the environment and be a positive asset to our communities.

138 Targeting vector control interventions by defining WNV human risk in space and time

Dominick Ninivaggi, dominick.niivaggi@suffolkcountyny.gov, Scott Campbell, Tom Iwanejko, Ilia Rochlin

One of the biggest challenges for effective control of West Nile virus (WNV) outbreaks remains the unpredictable nature of where and when, or even if, human exposure may occur on sub-County geographic scales. Interventions, especially adulticiding, must be targeted to high risk areas and timed to occur before the human exposure. However, entomological data may not be sufficient to determine where and when that will be, especially in the early season before data accumulates. In many areas of the eastern US, the relationship between the entomological indicators of human risk such as minimum infection rates (MIR) and WNV disease is unclear. The data is often too uncertain at highly localized geographic scales to properly direct

operational mosquito adulticiding. We developed a simple decision making algorithm to define WNV human risk in space and time based on historical human disease data and robust county-wide entomological indicators. The procedure can determine a high WNV risk year based on early season activity in mosquitoes. Preventative adulticiding can then be targeted to the previously mapped WNV geographic "hotspots" and timed prior to the peak human exposure. During low to medium WNV risk years, human involvement, as measured by case locations, is typically too scattered and random to justify adult control measures in any particular area. This targeted approach also has environmental benefits by directing adulticiding efforts only to those areas where it is likely to make a difference and reducing or eliminating adulticide use in the majority of years when WNV activity is low. Similar approaches can be developed by other local vector control jurisdictions by analyzing multi-year data on entomological indicators and human disease.

Next Generation Application Technology Symposium

139 A new field-tested compression sprayer for indoor residual spraying

Peter Obenauer, yry3@cdc.gov, Joe Diclaro II

Indoor residual spraying (IRS) with insecticides is an important control measure to lower malaria infection by targeting the malaria vector in Sub-Saharan Africa, *Anopheles gambiae* Giles. For over fifty years the manually operated compression sprayer has been considered the gold standard by the World Health Organization (WHO) for IRS operations. The JQSX-12 is a new innovative compression sprayer and was evaluated against the WHO gold standard and another commercial gas powered sprayer during an IRS operation in Liberia for its effectiveness as an alternative operational tool for indoor residual insecticide application. Our initial field assessment demonstrates the JQSX-12 to be a durable, efficient alternative, especially in regions requiring frequent application.

140 Investigations with misting systems, barrier treatments, and space sprays operationalized with a mobile pesticide app

Seth Britch, seth.britch@ars.usda.gov, Kenneth Linthicum, Robert Aldridge

US military operations in field conditions face significant negative impacts on mission readiness from disease-vector and nuisance populations of filth flies, mosquitoes, and sand flies. Although measures are in place to protect personnel in the field from these insect threats, experiences from recent US military campaigns in southwest Asia suggested that these measures were not adequate. One of the underlying causes is that pest management techniques, equipment, and pesticides have generally been developed in warm temperate conditions and not sufficiently tested in the broad range of environments occupied by US military activities. For ten years, through the Deployed War Fighter Protection Program (DWFP), we have conducted a series of trials in multiple environments and ecological zones against a range of medically important target insects, using an array of combinations of pesticides, application equipment, and techniques. From this work we have developed a series of recommendations for misting systems, barrier treatments, and ground and aerial space sprays that are effective in specific environmental conditions against specific target insects. To make these recommendations immediately operational we are developing a mobile Pesticide App that provides up to date, enhanced, and interactive guidance to both military and civilian public health pest management planners, applicators, and leaders.

141 Eave tubes with electrostatically coated liners for delivering insecticides against endophilic mosquitoes

Matthew B. Thomas, mbt13@psu.edu, Bart G.J. Knols

Control of malaria vectors is being increasingly challenged by the spread of insecticide resistance. Here we present a novel strategy to help combat this challenge. The approach has two key components. First is the use of netting treated with an electrostatic coating that binds insecticidal particles through polarity. The electrostatic netting can hold small amounts of powdered insecticides and results in enhanced bioavailability upon contact by insect vectors. Second is use of 'eave tubes' as a delivery mechanism. African malaria mosquitoes have a strong preference for entering houses at night through the eaves (the gap between the roof and the walls of a house). The eave tube approach involves blocking the eaves and inserting tubes that act as 'chimneys' to funnel human odors and create focal points for mosquitoes to attempt to enter the house. Once inside the tubes, the mosquitoes encounter the treated netting and pick up the powdered insecticide. Eave tubes are simple tools that can be fitted to many types of houses. They work passively (i.e. not requiring daily activity from house owners) and, being beyond the reach of house occupants, they also enable the use of (bio)pesticides that would not be allowed on walls or bednets, creating new options for using resistance breaking compounds. Progress, challenges and next steps for the eave tube strategy will be discussed.

142 Incorporating a low-cost imaging system into mosquito abatement operations

Clint Hoffmann, clint.hoffmann@ars.usda.gov, Chenghai Yang, Brad Fritz

Aircraft play a significant role in the successful completion of many mosquito abatement operations. Besides spray missions, these aircraft provide a readily available and versatile platform for airborne remote sensing. Although various airborne imaging systems are available, most of these systems are either too expensive or too complex to be of practical use for aerial applicators. The objective of this study was to develop a low-cost, user-friendly imaging system that can be easily installed on existing aircraft and operated by pilots and other supporting personnel. The imaging system was assembled using off-the-shelf electronics and consisted of a digital camera to capture color images, a GPS receiver to geo-tagged images, a video monitor to view live images, and a remote control to trigger the camera. The camera was attached to the landing gear and/or step of the aircraft and the GPS unit and video monitor were installed in the cockpit. The parameters of the camera were optimized to acquire images under various altitudes, speeds and ground cover conditions. Geo-tagged images taken from individual sites or large areas were viewed and stitched together using free and inexpensive software. Users can assemble such a system and deploy it to readily identify marshes, small pockets of standing water, and other habitat that may be conducive to mosquito production.

Disease Vector III

145 Spatial-temporal patterns of mosquito-borne bunyaviruses in the northeastern US

Theodore Andreadis, theodore.andreadis@ct.gov, Philip Armstrong, John Anderson

Cache Valley and Jamestown Canyon viruses are mosquito-borne bunyaviruses that are enzootic throughout much of temperate North America. Both viruses cause human disease and appear to be common in areas where the virus is enzootic. However, human infections are largely under reported. The primary reservoir and amplifying vertebrate hosts are unknown, but white-tailed deer, *Odocoileus virginianus* have been incriminated based on experimental infections and the prevalence of neutralizing antibodies in wild populations. These viruses have been found in a diverse array of mosquito species but the principal vectors appear to vary widely. The spatial and temporal patterns of virus activity in mosquitoes as a function of mosquito abundance, land

use characteristics, and vertebrate host distribution and abundance will be presented based on intensive mosquito and arbovirus surveillance conducted throughout the state of Connecticut over a 16-yr period, 1997 through 2012.

146 Update on Heartland virus (Bunyaviridae: Phlebovirus): a human pathogen transmitted by ticks

Harry Savage, hms1@cdc.gov, Marvin Godsey, Nicholas Panella, Kristen Burkhalter

In June 2009, two 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 (HRTV). 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 was 1:500 over the 2012 study period. In 2013, we conducted field studies in NW Missouri at sites that yielded virus in 2012, at a 2009 case-property that did not yield virus in ticks during 2012, and at properties owned by 3 new HRTV cases detected by a hospital surveillance system. Infection rates in nymphs of *A. americanum* collected in 2013 were significantly higher than in specimens collected in 2012. In addition, HRTV was detected for the first time in adults of *A. americanum*. Virus was detected on the properties owned by both of the 2009 patients, and on properties owned by two of three new patients. Discussions with case-patients indicated that exposure to ticks from farming, land maintenance activities, and recreational activities such as mushroom collecting and turkey hunting are risk factors for infection.

147 Transmission of Heartland virus (Bunyaviridae: *Phlebovirus*) by experimentally infected *Amblyomma americanum* (Acari: Ixodidae)

Marvin Godsey, mjg9@cdc.gov, Kristen Burkhalter, Angela Bosco-Lauth, Mark Delorey, Harry Savage

Heartland virus (HRTV) is a recently described cause of human illness in northwestern Missouri and Tennessee. After studies conducted in 2012 implicated *Amblyomma americanum* (L.) as a vector of HRTV we initiated experimental studies to assess the vector competence of this species. Larval and nymphal ticks were immersed in high-titered suspensions of HRTV, and then were tested for virus at various intervals post-immersion. In later trials larval ticks were immersed in HRTV followed by engorgement on a rabbit. A subset of post-molt nymphs was tested for HRTV to estimate the transstadial transmission rate. One group of nymphs was co-fed with uninfected colony larvae to assess non-viremic transmission, while additional nymphs were fed on a rabbit and allowed to molt to adults. Male and female ticks fed and mated on a rabbit and females were allowed to oviposit. Male and spent female ticks were tested for HRTV, and offspring of infected females were tested to assess vertical transmission. Initial trials indicated efficacy of the immersion technique. Transstadial transmission from the larval to nymphal and adult stages was documented. Transmission by co-feeding occurred but at a low level. Vertical transmission of virus to offspring of infected females was observed. Serologic conversions (without viremia) in rabbits indicated oral transmission of HRTV. Our data suggest that *A. americanum* is a competent vector of HRTV, and that vertical transmission may be an important mechanism of virus maintenance in the environment.

148 Zoonotic disease in a peripheral population: Persistence and transmission of *Leishmania major* in a putative sink-source system in the Negev Highlands, Israel

Gideon Wasserberg, g_wasser@uncg.edu, Ruti Berger, Alon Warburg, Laor Orshan, Burt Kotler

Populations at the edge of their geographic distributions are referred to as peripheral populations. Very little attention has been given to this topic in the context of persistence of infectious disease in natural populations. In this study, we examined this question using zoonotic cutaneous leishmaniasis (ZCL) caused by Leishmania major in the Negev Desert of Israel as a model system. Here, we suggest that the regional persistence of Phlebotomus papatasi populations and L. major transmission in the Sede Boqer region could be explained through processes akin to sink-source and/or mainland-island metapopulation dynamics. Given its potentially enzootically superior ecological conditions, we hypothesize that the Zin Valley ecotope constitutes the "mainland" or the "source" patch for the Sede Boqer area where L. major transmission is persistent and resistant to local extinctions (die-outs) whereas the local sand fly populations on the Zin Plateau ("island patch" or "sink patch") are more prone to local extinctions. Between 2007 and 2008, we trapped sand flies and sand rats in the two areas and compared sand fly abundance and L. major infection prevalence in both. In both 2007 and 2008, sand fly abundance was high and continuous in the Zin Wadi but low and discontinuous in the Zin Plateau. Infection prevalence of sand rats was significantly higher in the Wadi (13%) compared with the Zin Plateau (3%). Minimum infection rate in sand flies did not differ significantly between the two areas. Overall, our results are consistent with the premise that the Zin Valley population is relatively robust in terms of L. major transmission, whereas transmission is potentially more tenuous in the plateau.

149 Overview of imported cases and outbreaks of mosquito-borne arboviruses

Roberto Barrera, rbarrera@cdc.gov

Public health has been impacted by the emergence of exotic vector-borne pathogens during the last decade and trends suggest that these events may intensify. There are two main transmission cycles of interest: zoonoses and anthroponoses. In the first, people are infected by the bite of bridge vectors carrying zoonotic arboviruses that circulate between non-human vertebrate hosts and vectors in enzootic areas. West Nile virus invasion demonstrated that New World ecosystems are receptive to exotic zoonotic arboviruses. The geographic expansion of zoonotic arboviruses is cause of concern (Japanese encephalitis, Rift Valley viruses). In the second case, arboviruses cycle between people and domestic mosquitoes in urban areas without the intervention of enzootic cycles. The main arboviruses of concern are already present in the Americas (dengue, chikungunya, yellow fever, Zika). There is also concern about the urbanization of American enzootic arboviruses such as Mayaro and Venezuelan equine encephalitis. Dengue importation into the USA has been recorded for a long time and several events of local transmission have occurred in receptive areas (Texas, Florida, and Hawaii). The recent expansion of chikungunya into the western hemisphere affects imported cases and a local outbreak was already recorded in Florida in 2014. Other concerning issues are the apparent resurgence of*Aedes aegypti* in Florida and its ongoing geographical expansion in California, along with the invasion of *Ae. albopictus* and *Ae. notoscriptus*. Vector surveillance and control has focused on zoonotic arboviruses in the USA, but the increased introduction of anthroponotic arboviruses requires focusing more on urbanized areas inhabited by container *Aedes* mosquitoes.

150 Experimental perturbations of *Culex* spp. Mosquito productivity and its potential impact on West Nile virus transmission

Joseph McMillan, jrmcmil@emory.edu, Andrea Lund, Daniel Mead, Rebecca Park, William Koval, Uriel Kitron, Gonzalo Vazquez Prokopec

Larvicides are a common tool for controlling *Culex* mosquitoes in urban road-side catch basins. However, the ability of this practice to reduce adult mosquito populations and mosquito-borne

pathogen transmission is seldom studied. We conducted a field experiment to determine the impact of larvicide applications in road-side catch basins on: *Cx. spp.* larval abundance, pupal productivity, and measures of West Nile virus (WNv) amplification. During July and September 2015, 36 catch basins within Grant Park (Atlanta, GA) were treated weekly with a *Bacilluis thuringiensis israelensis* (Bti) larvicide. 7 basins within the park were sampled before, during, and after the application period to monitor the effect of the larvicide on *Cx. spp.* breeding populations. Gravid and CDC light traps were set throughout the park to monitor the effect of larval control on adult populations. Blood samples from the park's avian reservoir population were collected to measure WNv activity. All collected *Cx. spp.* adult females were tested for WNv using virus isolation. These methods were paired with unmanipulated collections from Grant Park in 2014 as well as collections from an un-treated park in 2015. Before-After Control Intervention (BACI) analyses showed that the application of larvicides effectively reduced *Cx. spp.* breeding populations in Grant Park between 2014 and 2015 as well as between the 2015 pre- and post-intervention periods. BACI analyses of *Cx. spp.* population abundance between Grant Park and the untreated park were not significant, suggesting that the effective control of*Culex* mosquitoes with larvicides must consider the importance of other factors such as extreme weather events, drainage patterns, and non-catch basin breeding sites.

207 Utilizing a customized mobile field data management application for mosquito larval control and surveillance

Tim McGonegal, tmcgonegal@pwcgov.org

Our staff provides mosquito and forest pest monitoring and control services throughout Prince William County. Three years ago there was an effort to provide a more systematic approach to collecting and managing our large amounts of field data. The old system of collecting data on paper forms, followed by entries into multiple databases, was becoming inefficient and ineffective. After analyzing the data collection process, staff determined that an electronic data entry device that stored data on a server in the Cloud would be able to resolve many of the problems associated with data entry and reconciliation.

We developed a mobile application utilizing tablets to streamline our daily inspection workflows. Inspection sites are available via a GPS-enabled map interface. The application is user-friendly and allows the field user to complete an inspection using customized data forms. The application allows citizen requests to be assigned to specific field users within minutes of the citizen calling. The application also has an "in-office" component that allows us to produce reports, graphs, charts, and real-time field summary data.

This presentation will highlight the systems features and show how this new system has increased our work productivity and customer service. I would like to do a live demonstration of the application right from the tablet to quickly show the features in real time.

Adult Control IV/ Equipment

151 Revisiting aerosol sampling reading methods

Derek Drews, ddrews@clarke.com, Jacob Hartle

Currently there are two widely used methods of aerosol sampling involved in ground and aerial adulticiding: (1) the use of a 3mm acrylic rod or (2) a 25mm Teflon coated glass slide. Our objective was to determine a method of droplet sampling that mosquito control professionals could use to establish equivalence in all adulticide applications. The 3mm rod is said to have a higher collection efficiency due to increased rotational speed, while the 25mm slide is said to be more efficient at collecting larger droplets but decreased droplet densities. Previous research has

shown the outside edge (5mm from edge) of the 25mm slide has much higher droplet densities then the remaining 20mm of the slide. Reading this outside edge of the 25mm slide shows that it is comparable to the 3mm rod in droplet collection efficiency, while maintaining its larger droplet collections. This method can allow sampling with either slide or rod and have comparable data.

152 Droplet penetration, characterization, and efficacy of aerial applications of Dibrom ULV adulticide in a desert environment

Ary Faraji, ary@slcmad.org, Nelson Long, Nadja Mayerle, Gary Hatch, Robbie Allen, Malcolm Williams, Peter Connelly, Sammie Dickson

153 Measurement of aerially sprayed droplets through a canopy to recognize cloud and spray dispersion in common mosquito resting sites

Grifith Lizarraga, glizarraga@clarke.com, Jesse Julien

ULV aerial applications and their measurement have been a topic of debate since their inception. As technology moves forward and ULV applications are well-established spraying techniques, there is a greater need for methods that can measure and compare sprayed clouds through unique habitats and environments. This study was designed to measure how droplets from an aerially sprayed product behave throughout and when they hit the inside of a canopy at different heights. A pole with droplet impingers (spinners) installed at different elevations collected the dispersion of the cloud as it moved vertically and weather stations monitored microclimates within the canopy. This study will help improve proficiency of an aerial spray and how droplet density and droplet size will be filtered through a canopy and other areas that mosquitoes are both resting and active; it will guide and delineate how much product will, potentially, be suspended in locations that are difficult to penetrate.

154 45° or 0°: Efficacy of ULV sprayer nozzle discharge direction in urban Gainesville, Florida against *Aedes aegypti*

Peter Jiang, jiangy1@cityofgainesville.org, Muhammad Farooq

Ground Ultra-Low Volume (ULV) spray is one of the most common methods to control adult mosquitoes in the State of Florida. During the normal practice, ULV sprayer nozzles are directed 45° upward. However, an open field study to compare the effect of nozzle discharge direction last year showed that it was more efficient when sprayer nozzle was horizontal vs. 45° upward. In order to validate the results in the urban environment, a field study was conducted to compare sprayer 45° upward and horizontal nozzle orientations in the urban area within the City of Gainesville, Florida. Twelve cages of 20-25 *Aedes aegypti* were held at road edge, close to and in front of the house, close to and behind the house, backyard and extending to the next road up to 300 ft. in two rows perpendicular to spray line. Alongside each treatment cage, a set of spinners using 3 mm x 75 mm slide was deployed to collect spray for deposition. Our field trial results were not completely in agreement with open field study. However, 2 out 3 field trials showed that 24-h mosquito mortality was higher when nozzle was horizontal compared with 45° upward. Meteorological conditions such as wind speed, humidity and temperature may all have impact on the efficacy of ULV spray. More field studies are needed in order to confirm the best discharge direction of ULV sprayer nozzle in the future.

155 Effect of ground travel speed on dispersion and efficiency of truck mounted ULV sprayer against caged *Aedes aegypti*

Muhammad Farooq, mufarooq45@gmail.com, Jennifer Gibson, Lisa Drake, Mike Smith, Jeremy Anderson, James Cilek, Rui-De Xue

To optimize ultra-low volume (ULV) applications with a truck mounted sprayer, a field experiment was conducted to study effects of ground travel speed on spray dispersion indicated by spray deposition and mortality of caged adult female *Aedes aegypti*. Study was conducted in an open field in northeast Florida during summer 2015. Aqualuer®20-20 was applied at maximum label rate using a truck mounted Cougar® ULV sprayer at 5, 10, and 20 mph. Two rows of cages containing mosquitoes were placed at 0, 50, 100, 200, 300, and 400 ft. from the spray line, and 5 ft. above ground. A Florida Latham Bonds (FLB) droplet sampler was placed next to each cage to determine spray deposition. Control cages were deployed out of the spray zone. Applications for one of three replicates with each travel speed were made in a day. Knockdown at 10 min and mortality at 24 h post-application was recorded.
Results indicated that 20 mph travel speed provided better spray dispersal which reduced with reduction in travel speed. Complete mortality was recorded for spray line up to 300 ft. by 20 mph travel speed, and at 50 and 100 ft. by 10 mph. Mortality by 5 mph ranged from 21 – 97 % at all distances. Significantly higher deposition resulted from 20 mph up to 100 ft. compared to other speeds. At other locations, travel speeds did not affect deposition. Based on field observations, it appears that spray dispersal is improved at higher speeds due to greater deflection of spray, and better mixing of spray in air.
Disclaimer: The views expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U. S. Government.

156 Evaluation of three electric-powered handheld ULV foggers with Aqualuer 20-20 ` against *Aedes albopictus*

Mike Smith, msmithamcd@gmail.com, Kinsey Camelio, Jennifer Gibson, Lisa Drake, Rui-De Xue

Three electric-powered hand held ULV Foggers (Longray ULV Fogger Model 3600B with rechargeable lithium battery, Longray ULV Fogger Model 3600E with 110V or 220V AC electricity, and pump up Boston Fog Battery Motorized Fogger) were tested against caged *Aedes albopictus* under semi-field conditions using Aqualure 20-20™ (1:5 dilution) in St Augustine, FL. Mortality of the caged *Ae. albopictus* were analyzed after 24 hours to determine the success of a single application. All three foggers caused 100% mortality after 24 hours post treatment. Initial calibration of droplet size from all three foggers showed larger droplet sizes than what is stated in each fogger's operational manual. The Boston Fogger had the highest droplet sizes collected.

157 Oil versus water adulticide formulations and their interaction with equipment and each other

Gordon Morrison, Gordon.morrison@bayer.com, Kurt Vandock, Britt Baker, Jason Trumbetta

With the growing popularity of water based adulticides, mosquito abatement districts will switch more frequently between oil and water based products for wide area mosquito control. This presentation will address the following: current procedures and practices when switching ULV equipment from oil to water based formulations, the interaction between oil and water based insecticides when mixed together, and what happens to the flow rate and droplet spectrum of each formulation when no alterations are made to the application equipment.

Survey results will be presented on the current attitudes and procedures regarding oil and water formuations and how different applicators address switching from one formuation to

another. Pesticide labels very rarely address how different products react with each other. Visuals with descriptions regarding how different formuations react with one another will be discussed. Finally, results will be presented and recommendations made regarding how to properly alternate between oil and water based ULV applications.

158 Improved operations in mosquito control through innovation

Suzanne Bartlett, sbartlett@volusia.org, James McNelly

Innovations in Volusia County, FL have improved mosquito control operations. Today's challenges, including newly imported viruses and diseases, have pushed the District to reinvent its approach to service delivery. Staff is focused on implementing fresh ideas with a proactive approach to address the mosquito production in salt marsh, fresh water, rural, and urban environments for a truly integrated mosquito management program. Learn how implementing new equipment, innovative techniques, work processes, and web based software have resulted in improved efficiencies and effectiveness.

159 The 3 Rs of reducing pesticide packaging waste

Dave McLaughlin, dmclaughlin@clarke.com

This presentation will discuss survey results of 80 abatement programs and their views on disposal of pesticide packaging waste along with their reception to 3R packaging: Returnable / Reusable / Recyclable. Examples of existing programs as well as packaging options under development by Clarke also will be discussed. These will include both downstream and upstream packaging initiatives, and the challenges and opportunities they have created. The presentation may cause you to rethink what you are doing in your own program and what you may ask of your suppliers.

The Power of One Symposium

165 Starting with why – The art & science of public health

Jason Clark, Jason.clark@valentbiosciences.com

Imagine a world where everyone wakes up every day inspired to go to work. I believe that's true for us. Very few AMCA members would answer the question "What do you do?" with "I kill mosquitoes". Instead, responses typically revolve around our passion about protecting the public's health. Whether you're a mosquito abatement manager, vendor rep, a CDC scientist or student, if you're drawn to mosquito control, you quickly realize there's an art form inherent to accomplishing our goals. It's a delicate balancing act between ensuring we capitalize on this industry's limited resources/tools while embracing the latest science that supports our creative approaches to mosquito control. For major public health initiatives, public + private partnerships have been the key to this balancing act over the course of time. As such, we'll look to outline some of the more intriguing stories that unpack this idea.

168 A better mozzie trap: A twisted journey through my mosquito trap obsession

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

Australia is threatened by several exotic diseases carried by mosquitoes, including dengue, chikungunya, and Japanese encephalitis. I have been involved in the surveillance and control of these pathogens since 1994, and have been especially motivated by the need to develop improved mosquito-borne virus surveillance systems (ie., a better mozzie trap). I will discuss my journey

through a shedscape of mosquito traps as I have sought to make arbovirus surveillance safer, cheaper, and cleverer. This has involved eliminating logistical bottlenecks along the way, including the use of sentinel animals, the need for powered traps and batteries, the processing of thousands of mosquitoes, and potentially the requirement of carbon dioxide. Ultimately, we hope to utilise these traps as an efficient and inexpensive way to monitor for exotic mosquito-borne pathogens.

166 Been there, swatted that – traveling the globe through entomology

Jennifer Remmers, Jennifer.lee.remmers@gmail.com

The field of medical entomology is highly diverse, offering a myriad of opportunities for adventure. This presentation highlights some of the personal experiences of a medical entomologist who has worked throughout the world on a wide variety of projects for the US military and for industry. Topics to be discussed include humanitarian and operational support for the US Navy for a variety of vector and pest management activities, entomological oversight of aerial insecticide and herbicide spray missions for the US Air Force, malaria control program support for the oil and mining industry, and pesticide product development, testing, and registration for the pest management industry.

215 The invaluable nature of a long-term mosquito surveillance program and dataset

Lyric Bartholomay, lbartholomay@wisc.edu

The dynamics of mosquito populations and arbovirus activity in the state of Iowa have been monitored from late Spring through early Fall annually since the 1960s. This critically important public health service to the state is provided by the Medical Entomology laboratory at Iowa State University, with partners in the Iowa Department of Public Health (IDPH), the University of Iowa State Hygienic Laboratory, and local public or environmental health departments. New Jersey Light Trap (NJLT) collections, made from traps that run nightly through (approximately) late May to September, have been a fixture of the program. Some of these traps have been run in the same location for decades, and thereby provide an almost unparalleled ecological dataset with unique perspectives to be gleaned on the ecology and phenology of mosquitoes, and epidemiology of mosquito-borne disease, in the Midwest. These data have proven critical for decision-making processes for emergency mosquito mitigation measures during several historical flooding events in the state of Iowa. The NJLT network of traps, supplemented by volatile-baited traps for host-seeking and gravid females, facilitated the discovery and tracking of several invasive species of mosquito and invasive species of human and animal disease-causing arboviruses. Based on information from this surveillance program, we understand the mosquito fauna of Iowa to include 55 species from 8 genera. Mosquito-borne viruses detected from mosquito collections over the years include West Nile virus, St. Louis encephalitis, La Crosse, and Western equine encephalitis; additionally, viruses that are not known to be pathogenic to vertebrates, were also described because of the data yielded from this long-standing program.

Next Generation Application Technology

160 Design and role of pyriproxyfen autodissemination stations for dengue vector control

Yi Wang, ywangs@hotmail.com, Devi Suman, Isik Unlu, Kshitij Chandel, Gregory Williams, Randy Gaugler

Dengue and chikungunya vectors such as *Aedes albopictus* and are container inhabiting mosquitoes that prefer to lay eggs in multiple small to medium-sized containers in urban environments. Our data has shown that among these containers, the cryptic habitats were more preferred by gravid females for oviposition. This behavior created hurdles for conventional chemical control strategies which often had low penetration to these habitats. We demonstrated that an autodissemination station (ADS) could attract gravid females, contaminates them with high concentration of pyriproxyfen (20% on oil band and 60% on powder band), and force them to exit and transfer the IGR to subsequently visited larval habitats. The station efficacy was tested under field conditions using natural mosquito population during the mosquito season of 2015 against hotspot area in Trenton, New Jersey. The stations were deployed in the field with the rate of 8 stations per acre and total of 18 acres of plot was treated after the plot was identified as a hotspot (≥ 5 mosquitoes per trap) by BGS trap data. Another plot of the same size was identified as control plot. The egg, larval and adult populations were monitored before and after the treatment in both treatment and control plots. Sentinel cups were sampled weekly post-treatment for larval bioassay. Field efficacy of the station was measured as population reduction (eggs, larvae, adults) as well as pupal mortality by IGR-bioassay of field samples using 3rd instar *Ae. albopictus* under standard laboratory conditions. Our results for the season of 2015 will be presented.

161 Innovative indoor residual spraying (IRS) equipment for malaria vector control

John Clayton, john.clayton@micron.co.uk

Indoor Residual Spraying (IRS) is a key intervention in malaria control programs. The technique uses compression sprayers (capacity 10 -15 litres = 2-3 USG) to apply residual insecticides to wall surfaces. Use of pressure regulating valves that control nozzle output and harder wearing ceramic tips, have brought significant improvements to the quality of IRS operations, although variability between operators still exists. To eliminate this, an automated portable track sprayer has been developed for experimental use to provide more consistent spray deposits for laboratory evaluations of different insecticides. This unit controls the speed of the lance, spray distance and angle as well as spray output and results of recovery of spray deposits from wall surfaces discussed. While recent progress in IRS has focused on incremental improvements to the equipment and technique, the logistical costs of IRS operations is still high as it requires teams of spray men to be deployed often to remote villages. Recent studies in Africa suggest this can be as high as 50% of the total operating cost including insecticides and equipment. There is scope to improve deposition of spray deposits on wall surfaces through novel application techniques, and potentially improve efficacy and persistence of insecticides, particularly as new and more costly formulations are developed. To explore this further a prototype electrostatic sprayer was developed using ready to use (RTU) formulations of insecticide. Spray treatments of 3 insecticides were applied on different surfaces, and bioassays conducted to monitor insecticide efficacy over time. Preliminary trials demonstrated the potential to reduce spray volumes significantly and improve insecticide efficacy.

162 What is old, is now new: larviciding with thermal fog technology

James Cilek, james.e.cilek.civ@mail.mil, Jennifer Knapp, Christy Waits

The effectiveness of applying various larvicides using the hand-held IGEBA TF34 in open and cryptic environments against larval *Aedes aegypti* in semi-field and operational field situations will be presented. Recent studies with Vectobac WDG have shown complete larval control of 2-3rd instar, within 24-48 hrs, in treated containers placed in cryptic habitats in an urban tropical environment.

163 Development of a new modular aerial spray system and night application capability for the U.S. Air Force

Mark Breidenbaugh, mbreiden@kent.edu, Karl Haagsma, Seth Britch, Kenneth Linthicum, Robert Aldridge

The U.S. Air Force maintains a capability with the C130 aircraft to conduct aerial spray operations over large areas for controlling insects of medical importance. The current modular aerial spray system (MASS) is custom designed to support a variety of configurations from ultra-low volume space sprays for adult mosquito control to ultra-high volume deposition sprays for applying larvicides. However, the system is now 25 years old and many of the custom parts have become cost-prohibitive to replace. Congress has decided to fund a replacement MASS, taking advantage of the latest technological advances in the field including a larger volume payload, rotary atomizers, and night vision compatibility. This paper discusses the development of a new MASS and covers multi-year investigations with the C130 aircraft to determine the highest pesticide release altitude which still allowed for repeatable results regarding insect mortality.

164 Aerial operations utilizing NOTAR technology in Volusia County, Florida

James McNelly, jmcnelly@volusia.org, Suzanne Bartlett

Volusia County Mosquito Control (VCMC) has utilized two MD500E series helicopters since the early 1990's to perform aerial surveillance and control of more than 50,000 acres of tidal wetlands. Over the past two decades, mild winters have enhanced the establishment and growth of native and invasive vegetation. Restrictions to mitigate plant growth and removal have impacted the ability to maintain long established landing sites. The MD500N model incorporates NOTAR technology and eliminates the helicopter's tail rotor, which in turn eliminates the potential for tail rotor strikes and potentially catastrophic events. VCMC's decision to purchase the 500N was in part based upon the success of this aircraft's utility and use in Chatham County's Mosquito Control Program. The integration of the MD500N within VCMC's aerial program has enhanced the safety of personnel and the constituents of Volusia County, reduces noise and promotes a "good neighbor" relationship within the community surrounding primary operations and has increased the visibility of VCMC's reinvigorated aerial mosquito surveillance and control program in central Florida.

The Power of One Symposium

169 Vectors from Iowa to California: Diverse opportunities working at a state public health agency

Renjie Hu, renjie.hu@cdph.ca.gov, Mark Novak

Employment at a state health department may offer opportunities to work with a variety of vector-borne diseases and related public health issues. At the California Department of Public Health, in addition to mosquito-borne diseases, other program areas include tick-borne diseases, plague, hantavirus, and vector control technician’s certification and training. These program areas provide opportunities to work collaboratively with colleagues from local vector control agencies, academia, local health departments, other state agencies, and federal agencies such as the CDC, US Forest Service, and National Park Service. The CDPH co-presenters will highlight some of the challenges and opportunities of working on a broad spectrum of issues related to vector-borne diseases in California.

167 A foundation in medical entomology could be the key to dream opportunities, including great collaborations, travel, and job offers anywhere in the world
Sara Erickson, erickson@wehi.edu.au

My career in medical entomology began with a job interview with Wayne Rowley. As an undergraduate assistant, I was immediately impressed with the laboratory work environment and the inclusion of students. Our office and lab spaces were always buzzing (with science and fun energy). I completed my MS degree at Iowa State University, having skills in collecting and identifying native mosquitoes, rearing numerous colonies, and infecting mosquitoes with West Nile virus in the Level 3 labs. I moved forward with mosquito-based research at the University of Wisconsin-Madison for a PhD program with Bruce Christensen. My adventures continued with collecting mosquitoes in Egypt, infecting mosquitoes with malaria and filarial worm parasites, and building research collaborations in Papua New Guinea. At the completion of my studies, I moved to Melbourne, Australia to set-up and manage two new high-containment laboratories at the Walter and Eliza Hall Institute of Medical Research. This year marks the 100thanniversary for this remarkable, private, not-for-profit research institute, which has recently expanded their research capacity for investigating mosquito-borne and infectious diseases (including malaria, Dengue, HIV and tuberculosis).

170 Integrated mosquito management – why a medical entomologist can do this job
Jason Kinley, director@gcmad.org

Medical entomology can provide the experience and education necessary to be successful in a mosquito management program. The presentation will focus on different reasons why a medical entomologist is prepared for an integrated mosquito or vector management position and will share the different experiences the author has had over the course of an 18 year career. Content is targeted towards young professionals in the field that want to learn more about integrated mosquito management and how education in medical entomology helps generate success in the service of mosquito and vector control.

216 When medical entomology and sustainability collide
Lyell Clarke III

The field of medical entomology under Wayne Rowley's guidance has resulted in many different career paths. This talk will outline how Lyell Clarke, former Ph.D. student under Wayne Rowley, took his medical entomology education and his interest in a sustainable business model and guided the family business on a journey towards greener, more sustainable products and practices for the public health industry.

Using the Private Cloud and Mobile Platforms for Effective Data Management

177 Out of the Dark Ages: Then vs. now
Beth Carey-Kovach

Then versus now – How new software has brought Charlotte County out of the dark ages
This humorous rendition depicts the not-too-distant past of Charlotte County Mosquito Control's system of data entry as compared to now. By utilizing an integrated web and mobile platform,

built for Integrated Mosquito Management, Charlotte County has elevated their program to new heights by streamlining workflow, increasing staff efficiency and supporting timely decision making.

218 The development and implementation of GEOPRO Database at the Collier Mosquito Control District
Adrian Salinas

178 Global impact: International mosquito data management
Derek Wright

Reviewing the process of internationalizing one mosquito management data system; Mugla is a large and proactive district in the western Republic of Turkey. Their unique challenge of developing a new vector control program, while implementing a new data system took a very motivated team. This talk briefly describes the process and challenges of implementing a geographical system in a new land and an American system in a new language.

179 New developments in mosquito data technology
Walt Wilson

Managing the breadth of information that supports effective mosquito control has never been easy. New computing resources and mobile platforms provide the tools needed to collect and make sense out of diverse mosquito management data sources, and to satisfy growing requirements for extensive reporting and analysis. This session will highlight key technologies that are available today, and look ahead at technology evelopments that will impact your operations in the not-to-distant future.

Resistance/ Susceptibility

180 Monitoring resistance in *Culex tarsalis* and *Culex pipiens* collected from a single site over time
Jacob Hartle, jhartle@clarke.com, Paula Macedo

It is recommended that abatement districts test for insecticide resistance in mosquito populations at least 1-2 times a year to help plan their integrated pest management (IPM) programs. Traditional methods for testing resistance provide snapshots of a sample population in a particular time and place. But isolated testing might not reveal if patterns of resistance vary within a single site during peak population. Our goal is to evaluate the variability of the resistance profile of a population from a single site over time. Our approach is to (1) test mixed-age samples of *Culex tarsalis* and F1 generations from wild gravid *Culex pipiens* multiple times during the local peak population or season at a single collection site. (2) Evaluate the resistance profile of each sample using enzymatic assays namely: alpha-esterase, beta-esterase, acethylcholinesterase (iAChE), mixed-function oxidases (MFO), glutathione-S-transferase (GST), and protein along with the CDC bottle bioassay. The *Culex pipiens* collections will also be evaluated with genetic screening for knockdown resistance (kdr). Abatement districts could use these results to decide if testing 1-2 times a year accurately reflects resistance profiles over a season or would help direct control decisions.

181 Nationwide mosquito susceptibility screening against six active ingredients
Stephanie Richards, richardss@ecu.edu, Jo Anne Balanay, Melinda Fields, Kurt Vandock

Mosquitoes may develop resistance to specific active ingredients in insecticides after periods of repeated exposure. Thus, it is crucial that target mosquito populations in abatement districts be tested for resistance to confirm efficacy of control and inform management decisions. Eggs from different mosquito species (*Aedes albopictus, Aedes triseriatus, Culex pipiens, Culex quinquefasciatus*) were collected from *ca*. 20 mosquito abatement districts in five geographic regions (Northeast, Southeast, Southwest, Midwest, West) of the United States. Centers for Disease Control and Prevention bottle bioassays were used to determine susceptibility/resistance ratios to six active ingredients used in mosquito control programs. Preliminary results show variation in insecticide susceptibility between species, regions, and active ingredients. To our knowledge, this is the first study to evaluate insecticide susceptibility for several active ingredients across several regions during a single season. Results of this study will provide a baseline of insecticide susceptibility and insight into possible resistance occurring in mosquito populations in the United States. Variation observed in susceptibility/resistance ratios in mosquitoes collected from different regions may help us understand trends in resistance and, hence, the importance of routine resistance testing in mosquito control programs.

182 Evidence for resistance to bifenthrin by *Aedes aegypti* in the Florida Keys

Lawrence Hribar, lhribar@keysmosquito.org

Bottle bioassays and treated vegetation assays were used to challenge *Aedes aegypti* with bifenthrin. Locally collected mosquitoes from Marathon and Key West, Florida were used. Two strains obtained from colonies, one known to be susceptible to permethrin and one known to be resistant to permethrin were used for comparison. Mosquitoes from Marathon died more quickly than did those from Key West. Both died more quickly than did the permethrin-resistant strain but more slowly than did the permethrin-susceptible strain.

183 Assessment of spray efficacy and resistance status during a West Nile virus and Saint Louis encephalitis virus outbreak in Maricopa County, Arizona

Cassie Scott, gni6@cdc.gov, Janet McAllister, Kirk Smith, James Will, Steven Young, John Townsen

Caged field trials using *Culex quinquefasciatus* and *Aedes aegypti* were conducted to evaluate the efficacy of sumethrin, permethrin, and etofenprox based products on mosquitoes collected during an outbreak of West Nile virus and Saint Louis encephalitis virus in Maricopa County, AZ in 2015. For the sumethrin based product, overall mortality in the field trials was 79% for *Cx. quinquefasciatus* at 0.0022 lb/acre and 89% at 0.0036 lb/acre. Mortality at the mid label rate was less for *Ae. aegypti*, overall 66%. For the permethrin based product, the overall mortality was 89% for *Cx. quinquefasciatus* at 0.0035 lb/acre. Overall mortality for the etofenprox based product at 0.0035 lb/acre was 79% for *Cx. quinquefasciatus*. Subsequent testing of *Cx. tarsalis* and *Cx. quinquefasciatus* in the lab demonstrated varying degrees of resistance in the population to permethrin, malathion, sumethrin, and etofenprox.

184 Impact of insecticide resistance on paternity success in Aedes aegypti

Miguel Moreno-Garcia, Miguel.moreno_garcia@colostate.edu, Karla Saavedra-Rodriguez, William Black IV

In *Aedes aegypti* mosquitoes knockdown resistance to pyrethroids has been selected in many populations. While resistance confers a survival advantage, it can be associated with higher fitness costs. This is because the required resources used for insecticide resistance are also

demanded for the expression of other traits linked with survival and reproduction. This results in a trade-off between trait types. Trade-offs may place the resistant individuals at a competitive disadvantage with the susceptible individuals. To better understand this possible disadvantages we have undertaken to study how insecticide resistance affect male paternity success of *Ae. aegypti*. In this study we compared the paternity success between two males when they have access to one female. One male came from a strain (Vergel) that has been selected for 6 generations with permethrin. The other male came from a susceptible strains. We analyzed paternity using as a marker a valine to isoleucine replacement in codon 1016 (Ile 1016: with segregation as a recessive allele) that has been associated with permethrin resistance. Paternity was determined through the analysis of melting curves patterns of offspring. We also analyzed the number of eggs laid by couples form the same strain and combined couples (resistant-susceptible). Results showed that males from the susceptible strain had higher paternity success compared with males from the resistant strain. Couples from the susceptible strains laid more eggs than couples from the resistant strain or combined couples. Heterozygous couples laid more eggs than homozygous couples. This results corroborated the hypothesis that insecticide resistance had a reproductive cost.

185 Susceptibility of natural and synthetic molecules, capable of inhibiting the enzyme acetylcholinesterase in vitro, on enzymes involved in insecticide mechanism

Aurora Carreño, quimicaal@hotmail.com, Angela Palacio, Thalita Vieira, Mario Silva, Vladimir Kouznetsov, Jonny Duque

The discovery of molecule mechanisms that can influence different target sites in the insect, as those ones that affecting acetylcholinesterase (AChE) activity, play a key role in the generation of new insecticides. The objective of this study therefore was to determine the action of α-aminonitrile and *Cymbopogon flexuosus* essential oil (EO) moderated AChE inhibitors with previous reported larvicidal and adulticidal activities on biochemical biomarkers of *A. aegypti*. Based on published biochemical assay protocols, five different groups of enzymes involve in insecticide mechanisms were quantified on the third instar larvae: α- and β-esterase, mixed function oxidases, *p*-NPA esterase, glutathione S-transferases and AChE. Direct action of the molecules on AChE obtained from the homogenate of adults and larvae without pretreatment was also evaluated. A Rockefeller colony without treatment was used as reference. Total protein per larvae pool and mosquito was measured following Bradford's protocol. The analyses were carried out on a *Versamax* microplate reader. The enzyme activity results of the treated larvae were comparable with those found for the reference colony except *p*-NPA esterase activity that showed a decrease of 46% when the EO, at a concentration of 61.5 ug/mL, was evaluated. The percentages of AChE inhibition in adults were between 14.7 and 24.3%, whereas in larvae were between 12.8 and 26.1% when concentration between 20 to 83 ug/mL were evaluated. The action of the substances evaluated on the most important enzymes involved in detoxification and resistance processes for *A. aegypti* was determined, this gives an insight into the mechanism of insecticide action evaluated *in vivo.*

186 Characterization of resistance in Anopheles gambiae: Mechanisms and magnitude

Jeffrey Bloomquist, jbquist@epi.ufl.edu, Fan Tong, Aaron Gross

West African populations of the malaria mosquito, *Anopheles gambiae*, have evolved resistance to pyrethroids and carbamates. The Akron strain from Benin possesses several resistance mechanisms, including a G119S mutation in acetylcholinesterase that gives high levels of resistance to standard aryl carbamates and the L104F equivalent mutation in the voltage-sensitive sodium channel giving resistance to pyrethroids. We previously reported resistance in this strain

to pyrethroids and DDT (13-fold to 35-fold compared to the susceptible G3 strain), but surprisingly little resistance to etofenprox, a compound sometimes described as a "pseudo-pyrethroid." Synergism studies with the mono-oxygenase inhibitor piperonyl butoxide and the glutathione-S-transferase blocker diethyl maleate showed variable synergistic ratios. As expected, the Akron strain also expressed resistance to pyrethroids in the larval stage (14-fold to permethrin), and this life stage is more amenable to electrophysiological analyses of nervous system sensitivity. Studies to characterize sodium channel-based resistance at the nerve membrane level in the Akron strain are in progress.

Mosquito SIT Symposium and New Aerial Relase Options

187 How do you like your mosquitoes... All natural? With frosting?

Stephen Dobson, sdobson@uky.edu, Hanano Yamada, James Mains, Corey Brelsfoard

The ongoing problem of mosquito borne disease provides an impetus to develop additional methods for the control of invasive mosquito species and against the globalization of mosquito-vectored pathogens. In addition to the development of new active ingredients, there is need also to develop additional methods for delivering pesticides. Autocidal methods rely on the use of mosquitoes to 'self-deliver' pesticides and may provide a useful compliment to traditional application methods. Here, the results of recent field trials will be presented. The trials are based on the release of male mosquitoes that have been either 1) infected with a naturally-occurring bacterium "Wolbachia" to cause sterility in a targeted population or 2) dusted with pyriproxyfen, which is a powerful inhibitor of immature mosquito development. The Wolbachia method is non-GMO and categorized by the EPA as a microbial biopesticide. The Wolbachia method is species specific and has been developed for multiple mosquito species, including *Aedes albopictus, Aedes aegypti* and *Anopheles stephensi*. The pyriproxyfen dusting approach can be used alone, or combined with classical Sterile Insect Technique, Wolbachia and GMO approaches, to increase the overall impact of the introduced male mosquitoes. The different approaches will be discussed and contrasted, and their relevance to different mosquito control contexts, including areal delivery, will be discussed. The results of recent field trials will be summarized.

188 Develop a *Wolbachia*-based incompatible insect technique (IIT) for dengue vector control through a field trial

Zhiyong Xi, xizy@msu.edu, Xiaoying Zheng, Ziqiang Yan, Yu Wu, Wei Qian, Jian Zhu

Wolbachia is a maternally transmitted endosymbiotic bacterium that is estimated to infect up to 65% of insect species. The ability of *Wolbachia* to both induce egg sterility and pathogen interference makes it possible to develop it as a biological agent for control of dengue and malaria. We have succeeded in transfer of different *Wolbachia*strains into *Aedes aegypti*, *Ae. albopictus*, *Anopheles stephensi* and *Culex quinquefasciatus*, and established a stable association between those mosquito species and a novel *Wolbachia*, with either single, double or triple infection. In order to develop a method for using these strains for disease control, we have started a field trial to release *Ae. albopictus* males, which carry *Wolbachia w*Pip from *Cu. pipiens*, for population suppression in Shazai Island in Guangzhou China. With mass rearing capacity being developed, mosquitoes were released weekly from March to November 2015 to reach an effective ratio of released vs wild males. A significant suppression has been observed with zero egg hatched frequently detected in a number of locations. We will report our progress in field trial and discuss challenges to be addressed in order to deploy this technique for implementation in dengue control.

189 Species control through aerial application with SIT

Kurt Friedemann, kfriedemann@dynamicaviation.com

There is a growing epidemic surrounding mosquito-borne diseases that have been carried to virtually every corner of the globe. These diseases currently do not have a vaccine and therefore have been traditionally controlled by attacking the mosquito that bears and delivers that sickness. Typically these mosquitos have been controlled through the use of pesticides – but the technique has not been historically completely effective. Sterile Insect Technique has been a topic of discussion for years based on the success of organizations like the USDA controlling the Mediterranean fruit fly, but because of the delicate nature of a mosquito, traditional methods of sterilization were not viable. However, advances in technology have changed that landscape and a need to treat large scale areas has become a necessity. Here, the benefits of aerial application for SIT will be discussed as well as the necessary equipment, technology and lessons learned that will create and sustain a successful aerial SIT campaign. Since 1992 Dynamic Aviation has been involved in providing aerial SIT both within the United States and internationally and has amassed more than 270,000 flight hours as the recognized leader in the dispersal of sterile insects in the United States. Based on that experience, this discussion will revolve around that experience and expertise as it relates to potential future projects using SIT in mosquito control.

190 The age old problem of getting it in the right place at the right time

Derric Nimmo, derric.nimmo@oxitec.com

Almost all mosquito control strategies require you to get the control method in the right place, at the correct dosage and at the right time. Anything else is just a waste of time and effort. With insecticides it is getting into the breeding sites or onto the adults to have the desired effect. In sterile insect techniques (SIT) there is the advantage that the insect (usually males) actively seek out females. Even so, the release of millions of insects over a wide area and keeping them healthy and fit enough to compete with WT males is a challenge. Mosquito adults are fragile and when millions need to be released weekly it presents some unique challenges for any release device design. Oxitec has released over 100 million genetically engineered males so far and expansion into larger areas will require some novel and challenging developments in release systems. This presentation will discuss the challenges, some of the solutions currently in use and what is needed in the future for genetically engineered sterile mosquito release equipment and strategies.

191 Technology to safely release millions of treated male mosquitoes from the air to combat disease

Ralph Breslauer, rsb5779@gmail.com, Hanan Lepek

In this section of the Symposium we will be explaining the brand new break through technology that has been created by aeronautic engineers to enable the safe mass distribution of treated/sterile male mosquitoes from aircraft. You will receive a overview if this exciting technology as well as see results from recent trials validating the efficacy of the technology. This will include HD video of thousands of mosquitoes being released at very high speeds and then safely flying away. We will also lay the groundwork for how this the technology forms part of a complete offering that will help the world meaningfully address vector borne diseases in large or hard to reach areas.

Vector Mosquito Control Response to Suspect Human Cases Symposium

192 Public health response to invasive *Aedes* mosquitoes in California

Renjie Hu, renjie.hu@cdph.ca.gov, Marco Metzger, Vicki Kramer

Since September 2011, three invasive mosquito species have been discovered in California: *Aedes albopictus, Aedes aegypti*, and *Aedes notoscriptus*. Both *Ae. albopictus* and *Ae. aegypti* are aggressive day-time biting mosquitoes that are capable of transmitting several viruses including dengue, chikungunya, and yellow fever to humans. *Aedes notoscriptus* may also be able to transmit arboviruses such as Ross River and Barmah Forest viruses in Australia and is a known vector for dog heartworm. Dengue and chikungunya viruses are prevalent in many parts of the world and expanding with ongoing outbreaks in Asia, Africa and the Americas. In California, human cases of both dengue and chikungunya are reported in visitors and returning travelers from endemic areas with known outbreaks. In response to the discovery of these invasive *Aedes* mosquitoes and potential local transmission of the diseases that they can transmit, the California Department of Public Health developed a document entitled "Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Locally Acquired Exotic Mosquito-Borne Diseases Transmitted by These Mosquitoes in California". The purpose and objectives of the guidance will be discussed in detail during the presentation.

193 Florida Department of Agriculture and Consumer Services vector control response

Adriane Rogers, Adriane.Rogers@FreshFromFlorida.com

The Mosquito Control Section of the Florida Department of Agriculture and Consumer Services (FDACS) operates under the authority of Chapter 388 of the Florida Statutes. The section administers approximately $2 million in pass through funding and grants and provides science based technical assistance, certification, and training to mosquito control programs throughout the state. In addition, the section evaluates and registers new mosquito control products as well as negotiates regulatory hurdles to allow for experimental testing on unregistered mosquito control products or uses. As part of the technical assistance provided to local mosquito control programs, entomologists at the Department provide training in mosquito surveillance, identification, and mosquito control techniques, loan out equipment such as microscopes and adult collection traps, and respond to emergency situations by activating the Mosquito Control Incident Response Team (MCIRT) following hurricanes, tropical storms, or intense flooding. The FDACS MCIRT has been activated 7 times since 2001 and has organized the treatment of over 8 million acres in Florida.

194 Manatee County Mosquito Control District's response to mosquito-borne diseases

Mark Latham, manateemcd@aol.com, Christopher Lesser

Like many counties in Florida, Manatee County has an abundance of mosquito species capable of vectoring both endemic and imported arthropod-borne disease agents.
The District monitors for the presence and increased circulation of the endemic arboviruses (primarily SLEv and WNv) that occur naturally in the bird populations through the weekly blood-testing of 14 flocks of sentinel chickens. The District responds to positive samples by increasing mosquito control activities against the presumed vector,*Culex nigripalpus*, primarily in the form of aerial adulticiding in the general geographic vicinity of the positive bird. The use of larviciding as a proactive population control measure is not practiced due to the almost ubiquitous occurrence of this species in numerous habitats throughout the county. Only one locally-acquired human case (WNv) has occurred in Manatee County in the past 20 years.
The primary imported mosquito-borne diseases are dengue, malaria and now chikungunya. The local health department informs the District of suspect imported cases, typically two or three a year, providing the approximate location (within a city block) so that mosquito surveillance, premise inspection and source reduction can be conducted by District personnel. Due to increasing concerns, the District has been conducting research into the efficacy of *Aedes*

aegypti control methods and achieves excellent, if temporary, reduction of pyrethroid-resistant adult mosquito populations through aerial adulticiding of the organophosphate products, Fyfanon and Dibrom. Nighttime small droplet aerial larviciding of methoprene is also conducted in the vicinity of suspect imported human cases, targeting the *Aedes aegypti* larval container habitats.

217 Development of improved surveillance technology for *Stegomyia* mosquitoes
Daniel L. Kline

195 Anastasia Mosquito Control District's response to suspect human cases in St. Johns County, northeast Florida
Rui-De Xue, xueamcd@gmail.com

Anastasia Mosquito Control District (AMCD) of St. Johns County has an agreement with St. Johns County's Department of Health about address release of the suspected imported mosquito-borne diseases since 2012. AMCD used the following strategies to handle the vector mosquito control response to the suspected imported human cases. For suspected imported malaria, West Nile virus, and Eastern Enquire Encephalitis (EEE), we emphases on the treatment about 5 miles of diameter because of long distance flight of vector *Anopheles*, *Culex*, and*Culiseta* mosquitoes. For mosquito control response to dengue fever and chikungunya virus, we concentrate on the treatment about 1 mile of the diameter due to a short flight distance of the vector *Aedes* mosquitoes. The control response includes promotion of education, increase of surveillance and inspection, larval control, and application of adulticides by ULV spray, or thermal fogging, or barrier treatment based on the climates and vegetation at the suspected case area. These strategies and methods were successful to have limited the spread of imported mosquito-borne diseases in St. Johns County, Florida.

196 Lee County Mosquito Control District's response to suspected cases & outbreak of mosquito-borne diseases
Wayne Gale, gale@lcmcd.org

Diseases such as SLE, EEE, and WNV are considered endemic to Florida while others such as dengue fever and malaria are routinely introduced into the state by infected human travelers. In the last decade both malaria and dengue have been established temporarily in Florida and achieved local transmissions for a period of time. Additionally new and emerging mosquito borne diseases such as chikungunya and Zika virus are moving around the world through travelers. Florida has mosquito species that are competent vectors of these diseases and it is important to continually monitor mosquito populations, the diseases circulating in the environment, and local introductions of mosquito borne diseases by travelers. Early detection and appropriate mosquito control responses are key elements in preventing the establishment and possible spread of mosquito borne diseases in Lee County, FL. The District has an aggressive approach to disease prevention through active collection and testing of mosquito pools using PCR, testing sentinel chickens using ELISA and maintaining an active dialogue with local health officials for alerts to imported mosquito borne disease patients. The District responds to any positive sentinel chicken or positive mosquito pool with immediate mosquito control in the vicinity of the detection. Upon notification of a human case, larval and adult mosquito control surveillance is initiated and rapid control of both larvae and adult mosquitoes is completed in the vicinity of the case. Most recently the District has incorporated the capability of wide area aerial urban larviciding using a water dispersible *Bti* formulation to combat container breeding vector species.

197 **Mosquito-borne disease outbreaks and the Navy Entomology Center of Excellence**
James Cilek, james.e.cilek.civ@mail.mil

Previous response efforts and current capacity of the Navy Entomology Center of Excellence to respond to US mosquito-borne disease threats and transmission events will be presented.

198 **Response to mosquito-borne diseases in Florida by the Florida Medical Entomology Laboratory**
Roxanne Connelly, crr@ufl.edu

The University of Florida, Florida Medical Entomology Laboratory (FMEL) is a research, training and education center devoted to research on the biology, ecology, and control of mosquitoes which are important transmitters of disease or pest annoyances, giving special attention to the needs of Florida's mosquito control organizations. The laboratory is also a center to train in the entomological aspects of public health, veterinary science, sanitation, mosquito control, drainage and irrigation design, wetlands management, and other areas requiring knowledge of medical entomology. Faculty are continually in contact with mosquito control directors and surveillance coordinators to discuss real-time data from mosquito and sentinel surveillance programs and to provide a forum for discussion of thresholds and best practices for responding to local arbovirus transmission. In the event of detection of new arboviruses into Florida, the FMEL would likely respond as when West Nile virus first appeared in the state by conducting field experiments to determine local vector potential. The FMEL extension program provides opportunities for training students to analyze surveillance data and for mosquito control and public health agencies to meet with academia, industry, and other government agencies to address preparations for introduction of new pathogens into the state, or increasing cases of mosquito-borne diseases that are part of the Florida landscape.

Anthropod Vector Highlights Symposium

199 **Highlights in vector biology**
Elizabeth Andrews, elizabethsandrews@gmail.com

Reviewed in this presentation is a selection of the published literature on the biology of arthropod vectors of human disease from the 2015 calendar year. Manuscripts were chosen based on their potential impact to the field of vector biology. Several major groups of disease vectors, such as mosquitoes, ticks, sand flies, and lice will be discussed. Topics will be broadly reviewed and include species descriptions, phylogeny, genetics, behavior, physiology, ecology, and pathogen transmission. Emphasis will be placed on articles effecting control practices and the epidemiology of associated pathogens such as parasites, bacteria and viruses. The objective of this review is to synthesize the new literature across a breadth of vector biology topics into a manageable format for the listener.

200 **Highlights of vector control technology**
Whitney Qualls, w.qualls@med.miami.edu

Following the tradition of "Highlights Symposia" in other professional associations, the published literature describing vector control technologies in 2015 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 vector control technologies both from an academic perspective as well as from that of the grass roots technician.

201 Highlights of flea-borne typhus (Rickettsia spp.) disease research, cat flea control techniques, and abatement proceedings, 2015

Laura Krueger, lkrueger@ocvcd.org, Robert Cumming

The incidence of flea-borne typhus cases has increased significantly from 2005-2015 in the United States. In California, Texas, and Hawaii, vector control districts and public health agencies are tasked with providing education, surveillance, and controversial control options in order to reduce disease transmission. This presentation summarizes epidemiological and scientific research published in 2015 that elucidates the complex transmission cycle of flea-borne typhus and insecticide susceptibility of cat fleas (*Ctenocephalides felis*) to commercially available flea control products. It also includes a summary of interesting legal abatement proceedings and court findings concerning flea-borne typhus in California in 2015. Ground breaking manuscripts will be discussed in depth and interesting or bizarre findings will be noted.

Larval Control II

202 Supression of Aedes albopictus, the Asian tiger mosquito, using a "Hot spot" approach

Nicholas Indelicatos, nindelicato@mercercounty.org, Isik Unlu, Kim Klingler, Ary Faraji, Daniel Strickman

Recent changes in climate and human behavior have led to dramatic increases in the abundance and geographic expansion of invasive mosquito vectors such as *Aedes albopictus*. Although source reduction has been shown to be effective in reducing mosquito populations, thousands of back yards need to be inspected during door-to-door campaigns, which is labor intensive and expensive. We identified "hot spots" as high numbers (≥ 5 female and male*Ae*. *albopictus*) of adult mosquito populations at very focal locations. We tested if hot spot source reduction efforts were effective in reducing mosquito populations in the early summer season (June to July). Analysis of historical data from the study sites indicated the proportion of hot spots in the control site relative to the intervention site was much greater in 2011 when hot spot treatments were applied to the intervention site, compared to 2012, 2013, and 2014 combined, when no sites were treated (OR (95% CI) =3.9 (1.8, 8.5), Z=3.39, P<0.001). Hot spot treatments can be incorporated into existing integrated mosquito management programs to increase the effectiveness while reducing time, cost, and effort spent for methods such as door-to-door source reduction.

203 Role of biotic and abiotic factors in controlling strategies for the immature stages of mosquitoes

Shabab Nasir, flourenceshabab@yahoo.com

A series of environmental and biological factors affect the activity of mosquitoes and efficacy of controlling methods. For this purpose, ten different numeric factors (Concentration of oils, Power of hydrogen (pH) of water, Dissolved oxygen (DO), Time, Electrical conductivity (EC), Total dissolved solids (TDS)) and categorical factors (Life stages, Water grades, Temperatures, oils) were studied. The effect of all these factors was studied on the mosquito's immature stages at two levels (low and high). These factors were screened out with the help of screening designes and significant factors (temperatures, time, life stages, concentrations and water grades) that increase the mortality level were identified. The significant factors were studied at five levels each, i.e., temperature (10 ºC, 22 ºC, 30 ºC, 34 ºC, 37 ºC), water grades (water with rich organic matter, tap

water, turbid water, sewage water and industrial water), life stages (1st, 2nd, 3rd, 4th instar larvae and pupae), time intervals (1, 3, 6, 12, 24 hrs) and concentrations (50, 100, 150, 200 and 250 ppm). Early life stages were found more susceptible than later ones and high mortality was observed with higher concentrations than lower. More mortality was observed in industrial water and sewage water due to acidic pH.

204 Flying into action: Helicopter larval treatments for MMCD

Jon Peterson, jonpeterson@mmcd.org

The Metropolitan Mosquito Control District (MMCD) provides a variety of services to 2.7 million people living in an area covering 2,900 square miles in the seven county Minneapolis and St. Paul, Minnesota metropolitan area. Over the past two seasons, we have had above normal amounts of rainfall during the summer months. This has led to multiple large broods of floodwater mosquitoes. This in turn has led to record amounts of larval treatments by helicopter. In 2014 we treated over 285,000 acres and in 2015 we treated over 300,000 acres by helicopter. We accomplished these larval treatments by becoming more efficient in our inspections, using all seven of the District's helicopters on the first day of the brood and by better communicating the helicopter needs between the satellite offices. We were also able to accomplish these treatments because of our efficient and accurate automated helicopter data system. By using the AgNav GPS in the helicopter and our own helicopter data processing system, we are able to quickly and accurately manage data from the treatments and use that information to plan and evaluate further treatment and communicate with the public.

205 Agnav flow controller for aerial larviciding

Lai Nguyen, lain@agnav.com

Just about 15 years ago, the aerial larviciding job required the pilot to be a master in multitasking between flying aircraft, turning spray on/off at the right locations, maintaining the right speed and swath for even application, and avoiding obstacles such as towers and power lines. Nowadays technology advancement can help achieve what were desired in the past. Aerial larviciding and mosquito control applications can have a sophisticated GPS system that can provide accurate guidance to the pilot to treat the right location, maintain correct swath, automatically manage the spray on/off precisely, and effectively control the flow rate based on speed of aircraft to maintain even application over the treated area.
To make the technologies work for you, AG-NAV recently developed a new product called the Granular Flow Controller to make larviciding more accurate and more cost effective to aerial applicators. This is done by replacing the uncontrollable slide gate of the IsoLair Broadcaster® granular applicators with a rotary gate driven by a motor. The spread rate control is done by adjusting the motor RPM based on the aircraft speed, swath width and target application rate. According to Lee County Mosquito Control District, the biggest advantage of the granular flow controller is the ability to change products in the field without manual recalibration. All materials to be applied can be calibrated with the flow controller ahead of time. The calibration curves are saved to a file in the GPS system. When changing material, the pilot just selects one of the preset calibrations. So far all test and real mission results prove the controller to be efficient and accurate; therefore delivering the right application rate even at various speeds.

206 Toward mosquito control with a green alga: Expression of genes from *Bacillus thuringiensis israelensis* in the chloroplast of *Chlamydomonas*

Seongjoon Kang, joonk@utexas.edu, Obed W. Odom, Saravanan Thangamani, David L. Herrin

Bacillus thuringiensis israelensis (Bti) produces a protein toxin that is highly specific for mosquito and black fly larvae; however, it does not persist in some environments, and cannot recycle. The green alga, *Chlamydomonas reinhardtii*, is an excellent food source for mosquito larvae. Moreover, chloroplast engineering in this alga offers genetic controls that are not found in other systems. The *Bti* toxin is a synergistic combination of 3 large Cry proteins (4Aa, 4Ba and 11Aa) and Cyt1Aa, although all of the proteins are toxic to larvae. To evaluate the potential for chloroplast expression of the protoxins, we are using the inducible system developed in the Rochaix lab, and codon-optimized genes for the Bti proteins, which also contain a C-terminal tag to enable antibody detection. Initially, we pursued the Cry genes: Cry4Aa was truncated at the C-terminus ($Cry4Aa_{700}$), while Cry4Ba and Cry11Aa were synthesized full-length. The genes were inserted into the chloroplast genome of the Ind41_18 strain, and homoplasmicity of the transformants confirmed by PCR. Western blots showed that each of the Cry proteins accumulated under induction conditions, with the following ratio: $Cry4Aa_{700}$>Cry11Aa>Cry4Ba (the range was 0.7%-0.07% protein). Bioassays with live algal cells indicated that the Cry11Aa and $Cry4Aa_{700}$ strains were quite lethal to mosquito larvae (*Aedes aegypti* and *Culex quinquefaciatus*). Additionally, we have recreated these strains in a wild-type *C. reinhardtii* host (therein providing constitutive production of the Cry proteins), which is also a necessary step toward using them for mosquito control.

208 Domestic inspections for the surveillance and control of Aedes albopictus and Aedes aegypti in Volusia County, central Florida

James McNelly, jmcnelly@volusia.org, Suzanne Bartlett, Jesse Julien

Volusia County Mosquito Control (VCMC) was founded nearly 80 years ago specifically to mitigate the impacts of salt marsh mosquito species. Peridomestic mosquito production and the prevalence of *Aedes albopictus*, coupled with the resurgence of *Aedes aegypti* and on-going importation of dengue and chikungunya virus, have necessitated a fundamental shift in VCMC's Integrated Mosquito Management Program. Door to door inspections targeting these mosquitoes are hardly unique to central Florida, and continue to challenge mosquito surveillance and control operations worldwide. The initiation of VCMC's domestic inspection program in 2012 was propelled through a collaboration with USDA-ARS and Rutgers University's Center for Vector Biology researchers. However, sustaining efforts that compel personnel to inspect private property is at best challenging, requires the support of myriad stakeholders and starts with a knock on a stranger's door. VCMC's domestic inspection program continues to evolve and provides invaluable educational opportunities for student interns and Volusia County citizens, and a foundation for disease response.