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Abstracts

Submitted papers, posters, and symposium presentations

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Celebrating the New Jersey Mosquito Control Association's 100th Anniversary

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The 2013 AMCA Memorial Lecture Honoree: Thomas Desmond Mulhern, 1908 – 1993

Thomas 'Tommy' Desmond Mulhern was born in Brooklyn, on June 15, 1908. He grew up in Fort Hancock, New Jersey, a coastal area with extensive salt marshes and mosquito problems that could only be described as biblical.

Tommy's extraordinary journey in mosquito control began in 1925, when he took a summer job as an Inspector for the Monmouth County Mosquito Extermination Commission, his first of three summers with the Commission. For transportation, the Commission provided him with a bike; his insecticide, kerosene, his application equipment, a garden watering can.

In 1926, Tommy graduated from Middletown High in Leonardo, New Jersey, where, unsurprisingly, he earned the Best Citizenship Award. He subsequently enrolled at Rutgers University in mechanical engineering and, thanks to Monmouth County, soon received the attention of mosquito control legend and Entomology Department Head, Thomas Headlee. Headlee added Tommy to his department in 1928 as a Drainage Engineer.

The focus of the Rutgers mosquito program when Tommy signed on was the saltmarsh mosquito, *Aedes sollicitans*. Tommy's early work, therefore, centered on applying his engineering skills to mosquito ditches. On his first day at Rutgers, Headlee charged him with rescuing a ditcher buried so deep that Tommy had to disassemble the machine and carry the parts to higher ground for reassembly. Tommy designed, built and evaluated ditchers, ditch cleaners, excavators, cranes, and anything that would dig or maintain mosquito ditches, always with an eye to make them cheaper, faster and lighter. Tommy's designs contributed to ditching most of the New Jersey and much of the eastern U.S. coastline. Tommy also supervised building a massive system of dikes and tide gates to reduce mosquito habitat. Here, he contributed several innovations including a 1944 'Inverted Siphon' drainage outlet that is still in use.

The area of research Tommy is best remembered for is pioneering adult surveillance methodology, particularly his invention of the New Jersey Light Trap. Tommy's trap, patented in 1938, became the 'gold standard' for adult surveillance for the rest of the century and is still in use all over the world. But his genius was less in the design of effective traps than it was in his resolute advocacy for using them to monitor mosquito populations. A strong case could be made for Tommy Mulhern as the 'father of mosquito surveillance.'

In 1935, Headlee organized a meeting of New Jersey mosquito control workers and their colleagues from nearby states to create the Eastern Association of Mosquito Control Workers (EAMCW). Headlee was elected president, and Tommy became the association's first secretary-treasurer, a position he held for 15 years. A controversial proposal was made in 1939 for the EAMCW to expand into a national organization. With a foot in both the academic and operational worlds, Tommy was a critical bridge between the different factions. He reconciled the opposing views and in 1944, EAMCW was rebranded to the American Mosquito Control Association.

In 1949, Tommy accepted a position as vector control specialist with the California Department of Health where he spent the rest of his career. The AMCA's business office, which had been based at Rutgers since 1935, relocated with him. Tommy's New Jersey experience was wielded in the formation of many new California mosquito control districts. Soon after Tommy's arrival, California's Central Valley was hit with major western equine and St. Louis encephalitis outbreaks. Tommy and the state of California responded by establishing the first state viral surveillance program. Tommy also developed early short courses covering mosquito biology, identification, spray technology and myriad associated topics. He was instrumental in the launch of the vector control operator's certification program, and in 1973 served as editor and principal author for California's first training manuals.

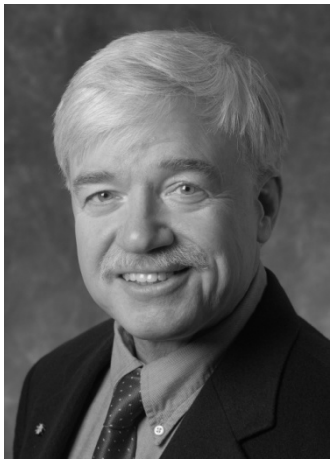
Tommy's mosquito control expertise was internationally recognized, providing frequent opportunities to serve as a consultant with the Navy, CDC, AID, WHO, PAHO and other prestigious organizations in Egypt, Iran, Central and South America, Saudi Arabia, China, Switzerland, and many other countries.

He served as a Trustee for the Fresno Mosquito Abatement District for twenty years. New Jersey and California both dedicated annual meetings to Tommy - New Jersey in 1994, and California in 2007 on the occasion of their 75th anniversary.

However, it was Tommy's role in AMCA where he left the greatest impact and a matchless legacy: as a founder, in his pivotal role in transitioning a small regional organization with an in-house bulletin to an association and journal with international stature, in starting the AMCA Newsletter, in incorporating AMCA as a nonprofit organization, and in his decades of service. Tommy filled every AMCA leadership post throughout the association's growth and development and held an astounding total of 28 years in key leadership roles: secretary-treasurer (1935–50), executive secretary (1950–52), president (1968–69), executive director (1974–78 and 1980–85). No one served AMCA longer, better, in more roles, or was more faithful than Tommy. The association has honored Tommy with every accolade possible: Medal of Honor (1973), Honorary Member (1979), Executive Director Emeritus (1985), Mosquito News December 1985 issue dedication, AMCA 50thaAnnual meeting dedication (1985). And today, he is honored with the final missing piece: the Memorial Lecture.

At age 76, Tommy suffered a heart attack, which effectively closed out his professional activities. When his beloved wife of 58 years Helen died, Tommy moved to a retirement home where he loved to tell mosquito control stories. In 1993, the Mosquito Warrior who began his career in Jersey's salt marshes passed away quietly in California's Central Valley, his journey complete.

The 2013 AMCA Memorial Lecturer: Randy Gaugler



Randy Gaugler was born in Fargo, North Dakota, in 1950. He graduated from West Fargo High in 1968, where he is a member of the Hall of Fame. After graduating, he enrolled at North Dakota State University at Bottineau on an athletic scholarship. Randy captained the wrestling team and won two individual conference wrestling championships. He was named in the 'Outstanding College Athletes of America', and in 1990 he was among the first athletes to be inducted and honored in the college's Athletic Hall of Fame. There, he also met Lumberjack cheerleader Cheryl Kester, whom he married in 1972. Randy received his B.S. from North Dakota State University, M.S. from North Carolina State University and Ph.D. from the University of Wisconsin, all in entomology. Following a 1979-1981 stint as Senior Scientist with the New York State Museum, researching the ecology and pathology of disease-transmitting black flies, Randy accepted an invitation in 1982 to join the faculty at Rutgers University.

Randy is currently a distinguished professor (Professor II) at Rutgers University in the School of Environmental and Biological Sciences. He also holds an appointment as professor in the Rutgers Medical School. His research has focused on parasites and pathogens of insects. He has made high impact contributions to our fundamental understanding of the host-parasite relationship between insects and nematodes, including foraging strategies, host finding, and the defeat of the host immune system. His pioneering development of molecular methods for insect nematology culminated in the first field release of a genetically-engineered insect parasite, an advance ranked as a Top Ten Historical Milestone by the New Jersey Agricultural Experiment Station. Randy's professional service includes serving on the editorial boards of six journals, the advisory boards of three companies, and various grant panels. He has been awarded more than $15 million to support his studies. His research is published in 230 papers and three books, and his body of work has received more than 7200 citations with h and i10 indices of 45 and 145.

Randy has taught numerous graduate and undergraduate courses, and lectured in forty countries on six continents. His present teaching responsibility is for General Biology. His teaching effectiveness, ranked at ratemyprofessors.com at 4.7 out of 5, reflects the deep satisfaction he derives from teaching.

Randy is the recipient of many national and international accolades for scholarship, most notably the prestigious Albert Einstein award (awardees include six Nobel laureates) from the Chinese Academy of Sciences, as well as the Recognition, IPM and L.O. Howard Distinguished Achievement awards from the Entomological Society of America. Randy has been elected a Fellow of the world's largest and most important scientific society, the American Association for the Advancement of Science, the highest honor the association confers. The Entomological Society of America, Society of Nematologists, and the Japanese Society for Science have similarly elected him a Fellow for "outstanding professional contributions". The Chinese Academy of Sciences elected him to the rank of Adjunct Member – a rare tribute for a foreigner, and last year awarded him with a Professorship for Senior International Scientists. In 2009, North Dakota State honored him with the inaugural Bolley Alumni Achievement Award, and in 2012 he received the Golden Service Award from Dakota College. A new species, *Mononchoides gaugleri*, has been named for him. Randy has also received many Rutgers honors, most recently the International Excellence Award and most significantly the Rutgers Board of Trustee's Excellence in Research Award.

In addition to his academic career, Randy took a leave-of-absence from Rutgers from 1993-1995 and was the research director for the biotech company Ecogen. He led an R&D effort with laboratories on four continents, including designing and staffing a laboratory in Italy from the ground up. During this time, he also devoted each Friday to working with his Rutgers team.

In 2006, Randy was appointed director of the 120-year-old Rutgers University mosquito program, guiding shifts in direction, focus and organizational culture that transitioned the unit into the Center for Vector Biology. In addition, this move led to a new emphasis in his laboratory on the highly invasive Asian tiger mosquito. Current research topics include pyriproxyfen auto-dissemination, IGR nontarget impact, oviposition behavior, host selection, carbohydrate utilization, and the biology of mosquito mermithids.

Randy has worked to see his research put to practical use. His innovations are the subject of multiple patents and licenses, and companies in the U.S., Canada, Europe, and Asia have commercialized his strains and production technologies. These efforts have resulted in eleven products and a culture medium.

Randy's scientific journey has provided extraordinary opportunities to explore the world. Expeditions, lectures, consulting, conferences and field studies have taken him to 80 countries, including fascinating locations such as Katmandu, Easter Island, Lake Titicaca, Xian, Siwa Oasis, Tasmania, Galapagos Islands, Azores, Rift Valley, Moldova, Macau, and the Amazon. These experiences have broadened his horizons and strongly influenced his outlook on life and career.

Randy and his wife Cheryl live in North Brunswick, New Jersey with their have two children, Kyle and Nikolas. They enjoy taking day trips to nearby New York City and Philadelphia for the museums, restaurants, theater and other cultural opportunities. They are presently enjoying Randy's third Fulbright award and living, working and exploring Egypt. Randy will return to Rutgers this June.

Oral Presentation Abstracts

Plenary Session

1 **If Gates gave you 10 million for mosquito research, what would you do with it?**
Bart G.J. Knols, bart@malariaworld.org

Surely a title like this gets us thinking. What are the real priorities, how can we come up with some really novel control tools, and how do we steer the innovation process? And perhaps also: What is hindering the development of radical new approaches to mosquito control? Is our environment conducive to radical thinking and radical solutions? And if not, how do we create it? In this talk I hope to convince you of the power of lateral thinking and how this can aid the discovery of new approaches in mosquito control. Was the discovery of Limburger cheese as an attractant for African *Anopheles* radical? Yes. Was it radical to train a sniffer dog to detect *Aedes aegypti* oviposition sites? Yes. Was it radical to turn our blood insecticidal by taking a magic pill? Surely, yes. These ideas stemmed from relatives, a lawyer, and a veterinarian, respectively. Not from peer medical entomologists. And all proved valuable. If we want to raise the global status of mosquito control as a primary means to curb disease transmission, then we need more drastic successes. Particularly in developing countries rife with malaria or dengue. Creative solutions are there, but to find them we need to leave our comfort zone and become 'out of the box' thinkers and do-ers. Challenging? Yes. Rewarding? For sure. So what would you do with 10 million?

1.1 **Managing mosquito-borne disease in Australia**
Cameron E. Webb, Cameron.Webb@swahs.health.nsw.gov.au

Mosquito-borne disease management in Australia faces challenges on many fronts. Increasing coastal urbanisation is bringing the community closer to productive mosquito habitats but environmental management of coastal wetlands is often in conflict with effective mosquito control strategies. Concerns regarding the indirect impact of broadscale mosquito control on local insectivorous bat populations have resulted in a reluctance of authorities to approve control programs. As a result, local authorities are turning to urban planning strategies in an attempt to minimise the risks to the community. Extensive flooding of eastern states in association with prevailing La Nina weather patterns has seen the re-emergence of the potentially fatal Murray Valley encephalitis virus. In addition, a virulent strain of Kunjin virus has had significant veterinary impacts across eastern states. Over the same period, the south west coast of Australia experienced some of the highest rates of Ross River virus infection and local authorities struggled to manage unusually large estuarine mosquito populations. As the east coast returns to hot and dry summers in association with La Nina weather patterns, concerns are being raised as to the potential risks associated with Ross River virus and Barmah Forest virus as estuarine habitats become more suitable for pest and vector species. In addition to these potentially enhanced conditions, major estuarine wetland rehabilitation projects are underway that hold the potential to significantly increase the abundance of local mosquitoes. The risks associated with dengue transmission remain in Far North Queensland. There is much debate surrounding the potential introduction and spread of *Aedes albopictus* into mainland Australia in combination with predicted southern movement of *Aedes aegypti*. Water-sensitive urban design strategies are intended to increase water conservation through water storage in new residential developments. In combination with a general water hoarding behaviour of the community during extended periods of below average rainfall, could we be creating conditions for potential dengue activity in our major metropolitan areas? These issues will be discussed and illustrated by local case studies.

Student Competition I

2 **The 24th student paper competition of the American Mosquito Control Association**
Brian Byrd, bdbyrd@wcu.edu

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

3 **Coating-embedded adulticide (CEA) and larvicide (CEL) for control of *Aedes albopictus* (Skuse)**
Ephraim V. Ragasa, evragasa@ufl.edu

Coating-embedded adulticide (CEA) and larvicide (CEL) were explored to control *Aedes albopictus*. CEA (0.7% permethrin) with simulated age of 35 months caused 100% mortality of adult females within 24-hr. The same CEA and CEL (0.01% pyriproxyfen) exposed to environment for 2,6 & 9 months caused 100% mortalities of adult females and prevented 100% adult emergence respectively. Lethal ovitraps with CEA, CEL or a combination of both were tested for effectiveness against gravid female populations.

4 **Overwintering biology of *Culex* mosquitoes and their role as overwintering reservoirs of West Nile virus**
Brittany Nelms, bmmills@ucdavis.edu

A major question is how WNV persists during winter at temperate latitudes when temperatures arrest viral replication and force mosquitoes into inactivity. *Culex tarsalis* and *Culex stigmatosoma* females entered and maintained a reproductive diapause during winter, ovarian follicles in most *Culex pipiens* complex females progressed to the host-seeking arrest stage and *Culex pipiens f. molestus* females remained reproductively active during winter. Virus was detected in overwintering *Culex* mosquitoes.

5 Evaluation of a new formulation of permethrin applied by water-based thermal fogger against *Aedes albopictus* in residential communities in St. Augustine, Florida

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

The efficacy of a new water-based formulation of permethrin against laboratory and field populations of *Aedes albopictus* was evaluated in the laboratory, semi-field experiments and residential communities in St. Augustine, Florida. 100% mortality was observed in 1h in the laboratory, 99% in semi-field experiments and significant reductions of adult *Aedes albopictus* and all mosquito species at 24h post treatment in residential communities

6 Fish in eastern Australian mangrove forest pools: potential mosquito control agents?

Lachlan F. Griffin, lachlan.griffin@griffith.edu.au

This presentation describes research identifying larvivorous fish populations in mangrove basins and evaluates their effectiveness as biological control agents of saltwater mosquito larvae (*Aedes vigilax* and *Culex sitiens*). Three native species and one introduced species were sampled in basin pools in two mangrove forests in eastern Australia. While all fish species fed on larvae, only one native species and the introduced one fed significantly enough to be considered potential control agents.

7 Dietary choices in *Aedes albopictus* are influenced by day-length

Alexandra Villiard, a.villiard@gmail.com

When day-length decreases in the Fall, temperate *Aedes albopictus* lay diapausing eggs which overwinter. We hypothesize that females reared in 10h light conditions will alter their diet to favor egg production over longevity compared to those reared in 14h light conditions. We offered blood and sugar meals concurrently every three days. 10h females more often skipped meals, while 14h females were more likely to blood-feed. This suggests that bite-rates per female may be altered in the Fall.

8 A mutation in the voltage-gated sodium channel gene in *Culex quinquefasciatus* from Harris County, TX

Mariah Scott, gni6@cdc.gov

Knockdown resistance is due to mutations in the voltage-gated sodium channel gene. We developed a melting curve assay that detected the Leu-Phe mutation in *Culex quinquefasciatus*. Allele-specific PCR detected the single nucleotide polymorphisms. Three different profiles were observed indicating homozygous resistant, heterozygous, or homozygous susceptible individuals. Insecticide resistance is a threat to public health, and detection is critical to the development of strategies for management.

Alternative Control Strategies I

9 Mosquito genetic control: back to the future

Graham B. White, gbwhite@ufl.edu

Genetic methods with the power to cut or even eliminate pest insect populations were first proposed during the 1940s but took decades to become reality. Before Knipling (1955) used sub-lethal irradiation to develop the sterile insect technique and applied it for eradicating the screwworm fly from North America, Serebrovskii (1940) suggested chromosomal translocations causing semi-sterility, but his concept was ignored until Curtis (1968) had the same idea. Both approaches led the way towards mosquito genetic control against vectors of malaria, filariasis and dengue, until political obstacles abruptly halted that momentum in the 1970s. Nowadays, genetically modified mosquitoes (GMMs with refractory genes, modified behavior, conditional lethals) can be readily produced and deployed for vector control and elimination. Instead of implementing integrated mosquito management continually, and struggling against insecticide resistance, it is feasible to extirpate the target species by genetic methods of increasing efficiency as their application progresses. This presentation briefly considers the operational feasibility, cost-benefits, manageable risks, and regulatory challenges of mosquito genetic control, with examples from current efforts against *Aedes aegypti and Anopheles arabiensis.*

10 RIDL: recent trial results and future prospects

Derric D. Nimmo, derric.nimmo@oxitec.com, Andrew McKemey, Camilla Beech and Luke Alphey

RIDL® is a new, environmentally friendly and innovative approach to the control of mosquitoes and many other insect pests. Mosquito-borne diseases, such as dengue fever, chikungunya and West Nile virus, are major and increasing international public health concerns. The 2 main vectors of dengue are *Aedes aegypti* and *Ae. albopictus*, both notoriously difficult to control with current control methods. In a RIDL control programme 'sterile' male mosquitoes (male mosquitoes do not bite or transmit disease) are released continually over a wide area to mate with the target pest population; death of progeny due to inheritance of the RIDL transgene leads to decline of the target population. Trials in the Cayman Islands in 2009 and 2010 tested and proved the technology could reduce an *Ae. aegypti* population, in that case by 80%, despite ongoing immigration from adjacent areas. Further trials in 2011 and 2012 in Brazil provided similar results. A regulatory pathway has now been determined in the US and we expect to perform a trial in collaboration with the Florida Keys Mosquito Control District in the near future. We will describe the results from the trials in Cayman and Brazil and discuss the potential for RIDL in the US.

11 Competitive ability of gamma-ray irradiated *Aedes albopictus* under differing release ratios
Odessa Madakacherry, o.madakacherry@iaea.org, Rosemary Lees, David Damiens, Sharon Soliban, Hanano Yamada, Marc Vreysen and Jeremie Gilles

To control populations of *Aedes albopictus*, an important disease vector, many regions are turning to area-wide integrated pest management with a sterile insect technique (SIT) component. The quality and competitive ability of mass-produced males compared to their wild counterparts is of concern as many of the methods associated with large-scale productions (e.g., mass, high density rearing, increased handling and irradiation) can affect their quality. SIT programs require that males be able to successfully compete with wild males for females. If the sterilized males released are less fit, whether due to lowered sperm counts, a reduced capacity to mate with multiple females, etc. than wild males, their number must be increased to compensate for the lowered mating ability. Lab-based studies have been undertaken to assess the competitive ability of sterilized *Ae. albopictus* males in competition with fertile males at varying sterile to fertile male ratios. In all treatments a 1:1 ratio of virgin females to fertile males in a range of cage sizes represented the natural population ratio to which an equal or increased number of sterilized males were added. Egg production and hatch rates are an indicator of the sterilized male's ability to vie for females. Data will be compared to existing field cage data to test if the lab trials are useful indicators of field conditions following a release. This will inform decisions as to the number of sterilized males to release in SIT programs.

12 A genetic sexing strain for *Anopheles arabiensis* vs. removal of females by spiking bloodmeals: a brief overview of method practicality
Hanano Yamada, h.yamada@iaea.org, Marc Vreysen, Jeremie Gilles, Sharon Soliban, Odessa Madakacherry, David Damiens and Rosemary Lees

The elimination of the blood sucking and potentially disease transmitting female mosquito is paramount for any mosquito suppression program that has a sterile insect technique (SIT) component. This can be achieved by biological, classical genetic or transgenic methods, all of which carry both advantages and disadvantages relating to male production efficiency and quality, reliability of female elimination, logistical practicality, or public acceptability. The creation, characteristics and treatment procedures of a genetic sexing strain for *Anopheles arabiensis* is described here, as well as alternatives such as separation of the sexes by spiking the blood meals and its feasibility for its use in large scale SIT programs for mosquitoes. Six toxicants added to blood meals were evaluated in terms of efficiency of female kill, effects on male survival and mating efficiency of the males after female removal. Preliminary results indicate that blood meals containing ivermectin eliminated >99% of the females in 2 d post-emergence, with no adverse effect on male survival. Both the virgin control males and experimental males were equally efficient in mating newly added virgin females. Based on the current results, an irradiation step will be added at pupal stage to evaluate the combined effects of treatments on the males produced. These results will show whether this method could be a temporary solution for sex separation until a better sexing strain has been developed.

13 The potential of mosquito release techniques for the control of mosquito-borne disease threats in the USA
Julian C. Entwistle, julian@xenexassociates.com

RIDL® and *Wolbachia* have been used in open release trials internationally to displace or control native mosquito populations. RIDL involves modification of the insect genome to cause sterility, whereas *Wolbachia* involves infection with a deleterious strain of this bacterium. In both cases open releases have been with *Aedes aegypti*, the principal global vector of dengue, which also transmits chikungunya and other diseases. The main mosquito-borne human infections in the USA are West Nile virus (WNV) and several encephalitis viruses. Potential threats include dengue, chikungunya and Rift Valley fever. *Aedes albopictus*, now widespread in the USA, is capable of transmitting many of these. RIDL® versions of *Ae. albopictus* have been developed and *Wolbachia* may also be used with this species, so these techniques offer the potential to control dengue and chikungunya. Several native *Culex* species also transmit WNV and the encephalitis diseases and some resident *Aedes* species are competent vectors of Rift Valley fever. *Wolbachia* is naturally present in *Culex* and prospects are good for obtaining a RIDL® form, so the potential exists for these techniques to be used against all the main vectors and to provide broad protection. The talk considers the issues involved and the future practicality of this.

14 A *Wolbachia*-based autocidal approach to control *Aedes albopictus*
James Mains, jimmymains@gmail.com, Corey Brelsfoard and Stephen L. Dobson

The Asian tiger mosquito (*Aedes albopictus*) is an invasive species and public health concern because of its ability to transmit medically important pathogens (e.g., dengue, chikungunya) and aggressive day-biting behavior. Despite intensive use of pesticides to manage this species, it has colonized much of the USA, 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 bacterium that is common in many insect species. Similar to sterile insect technique, this approach is based on the release of *Wolbachia* infected males, which cause a form of conditional sterility in the targeted populations. Presented here, small and large cages are used to examine for an impact of *Wolbachia*-infected males on populations of *Ae. albopictus*. Specifically, we tested for critical ratios of release to target males in which population impacts/elimination were observed. Our results indicate that release ratios of ~15:1 (release:target males) result in population elimination, with similar outcomes in both the small and large cage systems. The results in contained systems will be used to guide the design of open release trials, planned for the spring of 2013, pending EPA regulatory approval.

15 EPA approves experimental use permit (EUP) by University of Kentucky to test *Wolbachia pipientis* for mosquito control

Robert I. Rose, rirose1@juno.com and Stephen L. Dobson

The US EPA approved EUP No. 88877-EUP-1 June 15, 2012. This EUP is for sterile insect technique (SIT) control of *Aedes polynesiensis* on American Samoa where it transmits lymphatic filariasis. This EUP application by the University of Kentucky *Wolbachia* Project, supported by a grant from the Bill and Melinda Gates Foundation, was subject to the full EPA registration data requirements for microbial pesticides under FIFRA, except for performance data required for public health pest control, which is the reason for the EUP. The EUP was applied for under the Pesticide Registration Improvement Act, II, which requires 6 months for review and approval by the EPA and the EUP was approved in that time. This EUP was made available for public comment by EPA in the Federal Register and a few comments were received, but none of significance to merit federal disapproval. US EPA data evaluation report (DER) science reviews were provided by EPA consisting of human health and environmental effects risk assessments and these DERs were given to the Am. Samoa EPA and other concerned local government agencies. The University of Kentucky *Wolbachia* Project continues to communicate with Am. Samoa government agencies, addressing questions and concerns, including incorrect assertions by Am. Samoa EPA that *W. pipientis* is a chemical pesticide and new species, even though the federal EPA risk assessment analysis and DERs were conducted based on its identification as a microbial pesticide.

16 The mortal effect of photonic exposure on *Anopheles stephensi*

Emma Mullen, emma.mullen@gmail.com, Nathan Pegram, David Nash and Eric Johanson

Optoelectronic systems for three-dimensional, real- time field mosquito tracking and control are being developed for malaria eradication efforts. To be considered for widespread deployment, such systems must be constrained in cost and energy consumption. This is particularly challenging if a high power laser, small spot size, or expensive wavelength is needed to induce mosquito mortality. Laser diodes which find use in consumer electronics benefit from having similar constraints and are thus good candidates for inclusion based on price and efficiency. Some common wavelengths in consumer electronics were investigated for mosquito absorption and mortality. Two dosimetry systems were developed to deliver pulses of laser energy to *Anopheles stephensi* mosquitoes to investigate the effect these variables have on mosquito mortality in a controlled manner. The wavelength, power, and spot size were independently varied to investigate their component effects. One-d mortality measures are given for the 3 varied parameters. Based on these trials, the shortest wavelength (405 nm) most effectively killed mosquitoes, increasing the spot size by 60% had a significant impact on mortality, and the effect of laser power was shown to be nonlinear.

17 Development of an integrated push-pull system for the control of biting flies

Erica Lindroth, erica.lindroth@us.army.mil, Michelle Colacicco-Mayhugh, and Gabriela Zollner

Push-pull vector control strategies use a repellent compound to "push" arthropods away from hosts and an attractant to "pull" arthropods into traps. The objective of this study is to develop an integrated push-pull vector control system that is effective against mosquitoes, sand flies, and other arthropod vectors of disease. To this end, we have evaluated a number of commercially available repellent and attractant compounds against *Anopheles stephensi* Liston, *Aedes aegypti* (L.), *Phlebotomus papatasi* (Scopoli), and *Lutzomyia longipalpis* (Lutz & Neiva). Repellent and attractant compounds were first tested in a modified choice chamber system. With the exception of Off! Botanicals® with 10% PMD, all repellents exhibited significant repellency for all species tested. With the exception of the Lurex® Mosquito Magnet, no attractants exhibited significant attractancy in the choice chamber system. Based on the results from the choice chamber assays, products were selected for wind tunnel assays, which are ongoing. Repellent and attractant compounds selected from the wind tunnel assays will be tested in field trials in Africa, South America, and Asia.

Disease Studies I

18 West Nile virus in the United States, 1999-2012

Roger S. Nasci, rsn0@cdc.gov, Nicole Lindsey, Jennifer A. Lehman, J. Erin Staples, Marc Fischer and Lyle R. Petersen

The transmission ecology and epidemiology of WNV has changed since it was first detected in the US in 1999. During the "invasion phase" (1999 - 2004), WNV spread from east to west across the continent into new ecosystems. Some of the most extensive epizootics and epidemics we've observed occurred during this time, due largely to these being virgin soil epizootics where every bird in the population was susceptible. This, coupled with the combination of local conditions that facilitate virus amplification, resulted in the large epidemics of 2003-2004. Subsequently, WNV distribution expanded essentially everywhere across the country and established an "ecological equilibrium", becoming a part of the ecosystem and subject to the interplay of biotic and abiotic factors that produce periodic, focal population explosions in many different organisms. This presentation will summarize WNV epidemiology during the invasion and equilibrium phases, describe how the 2012 outbreak in north Texas was characteristic of this pattern, and discuss the potential for developing useful long-range and short-term forecasts of WNV risk.

19 Multi-faceted response to the resurgence of West Nile virus in New Orleans, LA 2012

Sarah Michaels, smichael@tulane.edu, Andrew Ruiz, Cynthia Harrison, Mieu Nguyen, Princeton King and Claudia Riegel

In April 2012, *Culex quinquefasciatus* populations were elevated and the earliest West Nile virus (WNV) positive mosquito pool was collected. By June, WNV activity in New Orleans was unprecedented, minimum infection rates

peaked at 14.62 and the virus was widespread. The New Orleans Mosquito and Termite Control Board used the guidelines in "Before the Swarm" (ASTHO) to design a multi-faceted response. GIS technology was used to integrate mosquito population and previous human case data to target areas at greatest risk of potential human transmission. Collaborative efforts with partner city agencies, New Orleans Fire, Police, Sanitation and Code Enforcement departments coordinated for a door-to-door public health awareness campaign in designated WNV risk areas. Additionally, areas with large numbers of abandoned properties, tire dumping and widespread water leaks were identified for source reduction and larvicide applications. Ground and aerial adulticide applications were 5 times greater than a typical year. A series of press releases were issued, focusing on the elimination of standing water and the use of repellants which was particularly important following Hurricane Isaac, as human exposure was greatly increased. The engagement of community partners assisted in our ability to respond to this crisis, and establishing these partnerships will allow for a coordinated response to other mosquito-borne disease emergencies in the future.

20 The impact of aerial adulticiding on West Nile virus activity in Suffolk County, NY in 2012
Dominick V. Ninivaggi, dominick.ninivaggi@suffolkcountyny.gov, Scott R. Campbell, Thomas Iwanejko and Ilia Rochlin

Aerial adulticiding is an important tool for reducing the transmission of West Nile virus (WNV), but measuring the impact of control operations is difficult, especially for operational programs because of resource limitations. In 2012, Suffolk County conducted 2, aerial adulticide operations with adjacent spray blocks in response to WNV. There were 4 virus surveillance sites in each block where WNV was detected in *Culex* spp. and 27 surveillance sites elsewhere in the county where virus was detected in *Culex* spp. but there was no adulticiding. This data set lent itself to a BACI (before/after, control/impact) analysis where virus activity before and after treatment in the treated blocks could be compared to control or reference sites for the same time frame elsewhere in the county. The results indicated that virus activity, measured as positive mosquito samples, declined more rapidly post-spray in the treated areas compared to the reference sites. The effect was more pronounced for the treatment where weather conditions were more favorable.

21 West Nile virus activity in central Texas during summer, 2012
Richard Duhrkopf, rick_duhrkopf@baylor.edu

Over 200 cases of West Nile virus were reported in central Texas during the summer of 2012 with almost half of the cases involving West Nile neuroinvasive disease and 8 deaths. Previously, the highest number of cases in this area for a single year was 7. The incidence of disease transmission was analyzed related to early year weather patterns.

22 Epidemiology of West Nile virus in New York City: analysis of mosquito data, 2002-2012
Marcia O'Connor, moconnor@health.nyc.gov and Waheed I. Bajwa

West Nile virus (WNV) first appeared in New York City (NYC) in August of 1999. Since then, the virus has found a permanent home in NYC and spread to every state in the continent. The infection shows up every year in the hottest months of July and August in NYC. In the last 14 yr, the City has experienced several outbreaks; the most significant was in 1999 when the infections resulted in 59 human cases, including 7 deaths. There was also, higher than normal activity in 2002, 2003, 2010 and 2012. Since 1999, the Department of Health & Mental Hygiene has collected a significant amount of data on this virus, its vectors and their role in the transmission of the virus in NYC. Recently we have analyzed these data; in this paper, we will discuss the role of biological and ecological factors associated with the outbreaks of the virus in NYC.

23 Use of time series models in West Nile virus surveillance studies
Juliet Kinyua, juliet.kinyua@tiehh.ttu.edu, Steven Presley and Kenneth Dixon

The Vector-borne Zoonoses Lab at Texas Tech University is involved in West Nile virus (WNV) surveillance in Lubbock, TX, collecting mosquitoes from different sites within the city bi-weekly using EVS™ CO_2 baited traps and screening pooled mosquitoes for *Flavivirus*. This has resulted in a databank of information related to mosquito population changes over time, accompanied by a consistent recording of WNV occurrence. Accordingly, we exploit this dataset to demonstrate how time series analysis models can be used to analyze long-term surveillance data. Using surveillance data collected from 2002 to 2012 in Lubbock, TX, the time series models describe trends and seasonal variations in vector populations from the region and how they affect WNV occurrence. By employing time series analysis techniques to investigate correlations between WNV occurrence and various environmental factors in order to describe structures of covariation which can then be used to estimate the probability of occurrences of WNV. The presentation's output can be used to inform and complement predictive disease models. Overall, the investigative and predictive robustness of time series tools applied to disease modeling efforts will be tested under various scenarios.

24 CDC Entomology branch and President's Malaria Initiative activities
Robert Wirtz, rwirtz@cdc.gov

This presentation will give an overview of current activities of the Centers for Disease Control and Prevention Entomology Branch, with a focus on the successes and challenges of vector control in 19 sub-Saharan African countries and the Mekong Region under the President's Malaria Initiative. Indoor residual spray and long lasting insecticide treated bed net programs will be reviewed, with an emphasis on: 1) monitoring and managing insecticide resistance, 2) bed net durability, 3) training and support of national malaria control staff, 4) product development

and field evaluation, 5) vector biology, and 6) outdoor transmission. A goal of this presentation will be to facilitate collaboration among US vector control programs in support of international capacity building and information management systems.

25 Identifying alternative arboviral testing methods in southeastern Virginia during a year of abundant eastern equine encephalitis and West Nile virus activity

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

Prior to 2009, Virginia state-run laboratories provided RT-PCR testing of mosquito pools for arboviral diseases including eastern equine encephalitis (EEE) and West Nile virus (WNV). In the fall of 2010 federal and state funding cuts limited mosquito testing to RT-PCR on pools confirmed positive by other testing methods. By the spring of 2012, Virginia state-run labs ceased all forms of mosquito testing. This prompted various regions of VA to search for alternatives. Most of southeastern VA started using VecTest™ in the spring of 2011 and in the fall of 2012 they started using VectorTest™; both are in-house antigen assays capable of testing EEE and WNV. In the 2011 season VecTest detected 1 EEE and 15 WNV positives, while in the 2012 season 160 EEE and 60 WNV were detected. At least 8 WNV positives were detected by VectorTest in 2012. As compared to previous years, 2011 had some of the fewest positives of EEE and WNV in the region while 2012 had some of the highest. Confirmation rates for VecTest EEE positives of *Culiseta melanura* and VecTest WNV positives of *Culex pipiens* were near 100%, while VecTest WNV positives of *Cs. melanura* were near 60%. The VectorTest positives were only at a 12.5% confirmation rate. Results with these alternative testing methods seem to be accurate in detecting EEE in *Cs. melanura* and WNV in *Cx. pipiens*; however, both testing methods have shown false positives for WNV in *Cs. melanura*.

Legislative & Regulatory Symposium I

26 An overview of AMCA legislative and regulatory issues: working with the 113th Congress

Ed Ruckert

Mr. Ruckert will present an overview of legislative and regulatory issues affecting AMCA members in the context of the new Congress and the Executive Branch.

27 Endangered species consultation: a new memorandum of understanding (MOU) between FEMA and the USFWS in Florida

Mike Hudon, m.hudon@irmosquito2.org

Hurricanes and tropical storms occur regularly in Florida, bringing with them epic rainfalls and subsequent hordes of mosquitoes that can pose a public health threat and hamper relief efforts. Whenever the Federal Emergency Management Agency (FEMA) is involved, there is a requirement that FEMA consult with the United States Fish and Wildlife Service to make sure their action will not harm endangered species. Storms and mosquito control responses are predictable and these agencies have consulted and agreed prior to any declared emergency that vector control measures if done according to protocol and in pre-designated areas will not harm endangered species in Florida.

28 Law and order: ESA

Angela Beehler, angela@mosquitocontrol.org

This study investigated the influence of interannual variations in temperature and precipitation on yearly West Nile virus (WNV) infection rates in the state of NJ. Drought conditions were associated with significant increases in yearly WNV infection rates at both the local and state levels. At the state level, early seasonal increases in temperature and decreases in precipitation were strongly correlated with increases in yearly WNV infection rates over a 9-yr period (2003-2011).

Student Competition II

29 Larvicidal activity of *Pinus densiflora* needle hydrodistillate constituents and related compounds toward wild *Aedes albopictus*

Dong Chan Lee, stjohndlee@gmail.com

The toxicity of red pine, *Pinus densiflora*, needle hydrodistillate and 29 constituents to 3rd instar larvae of wild *Ae. albopictus* was examined using a direct-contact bioassay. Results were compared with those of the currently used larvicides fenthion and temephos. Based on 24 h LC_{50} values, 3-carene (LC_{50}, 8.60 mg/L), limonene (LC_{50}, 10.34 mg/L), and thymol (LC_{50}, 10.65 mg/L) were the most toxic compounds. These compounds were more toxic than either fenthion or temephos.

30 Skewed sex ratio in rearing *Aedes albopictus* for sterile insect technique (SIT) releases

Odessa Madakacherry, o.madakacherry@iaea.org

The sterile insect technique (SIT) requires both massive releases of competitive males and the retention of some males for colony purposes. *Aedes albopictus*, the dengue vector targeted by SIT programs in Italy and Sri Lanka, can mate 6 times on initial sperm stock, indicating that colony sex ratio can be skewed in favor of females without affecting overall productivity. An experiment was conducted to determine the optimal ratio for colony cages and the results used as a standard in SIT projects.

31 Drought-induced amplification of local and regional West Nile virus infection rates in the state of New Jersey (USA)
Brian J. Johnson, brijo85@eden.rutgers.edu

This study investigated the influence of interannual variations in temperature and precipitation on yearly West Nile virus infection rates in the state of New Jersey. Drought conditions were associated with significant increases in yearly WNV infection rates at both the state and local levels. At the state level, early seasonal increases in temperature and decreases in precipitation were strongly correlated with increases in yearly WNV infection rates over a 9-yr period (2003-2011).

32 Population genetics of *Aedes japonicus japonicus* on the island of Hawaii
Andrea Egizi, egizi@eden.rutgers.edu

Despite being considered a temperate species, *Aedes japonicus japonicus* was able to establish a population on the island of Hawaii, where temperatures are warm year-round. To understand how *Ae. j. japonicus* is faring on Hawaii we examined its population genetics at low vs. high elevations where temperatures are warmer vs. cooler, respectively. Our study found evidence of ongoing bottlenecks at low elevation indicating *Ae. j. japonicus* populations in Hawaii are affected by temperature.

33 Bioassays to test area-wide applications of larvicides
George Condon, gccondon@hotmail.com

We optimized standardized procedures for bioassays to evaluate the efficacy and penetration of an area-wide low-volume application of the larvicide *Bacillus thuringiensis israelensis* (*Bti*) against *Aedes albopictus*, the Asian tiger mosquito (ATM), in residential neighborhoods. We present field and laboratory procedures to determine the ATM percent mortality after larvicidal applications to develop cost efficient ways to reduce adult ATM in urban/suburban residential neighborhoods.

34 Mosquito community differences compared according to livestock host
Margo L. Mire, mirem@lincolnu.edu

Livestock in rural environments are at risk of infection by mosquitoes as are humans. A survey of adult mosquito communities was conducted in central Missouri. Collections were made at different livestock holding locations. Statistically significant differences were found between groups and NMS results indicated a 5.5 stress value when assigning communities to host groups. This information will contribute to the enhancement of mosquito control efforts, protecting human and livestock populations.

Alternative Control Strategies II

35 Novel topical repellents for mosquito control
Robert Bedoukian, mmaher@bedoukian.com

Several topical mosquito repelling compounds have been developed. These molecules are based on naturally occurring chemicals produced by plants. They have low toxicity, low odor profiles, and low volatility, making them desirable alternatives to more traditional repellents like DEET® and para-Menthane-3,-8-diol (PMD).

36 Simulating sea level rise in a mangrove basin: implications for saltwater mosquito management
Jon Knight, j.knight@griffith.edu.au, Pat Dale, Shing Yip Lee, Jan-Olaf Meynecke and Lachlan F. Griffin

The presentation describes the implementation and results of a source reduction—habitat modification—experiment undertaken in an 18-ha mangrove basin in eastern Australia. The underlying rationale was to balance the objective of doing as little as possible to the ecosystem while doing enough to reduce the habitat suitability for mosquitoes. Although the original aim was to evaluate the effectiveness of hydrologic modifications, as a type of source reduction, the experiment also simulated sea level rise (SLR) by lowering the basin spillover elevation, similar to raising sea-level. This was done by modifying the tidal connection across a perimeter berm by digging a shallow channel (ratio of width to depth 3:1) between the tidal source (lake) and a pool landward of the berm. Tidal connections between pools within the basin were also established or enhanced by digging shallow channels or laying 300 mm pipes. This increased the frequency and duration of tidal flooding similar to the likely effect of SLR. The results showed that the impact of the modifications and of simulated SLR on the basin after one year was a reduction in mosquito production while improving water quality and enhancing fish habitat.

37 Life cycle, ultrastructure and molecular characterization of a novel microsporidian parasite from the invasive Asian rock pool mosquito, *Ochlerotatus japonicus*
Theodore G. Andreadis, theodore.andreadis@ct.gov, Hiroyuki Takaoka, Yasushi Otsuka and Charles R. Vossbrinck

Ochlerotatus japonicus (Theobald) is an invasive mosquito from East Asia that was first detected in the northeastern US in 1998. It has rapidly spread throughout eastern North America and has recently been found in central Europe. A novel microsporidian parasite, first discovered in 1980 from *Oc. japonicus* larvae collected from rock pools along the Okudake River in Japan has been re-isolated and evaluated for introduction in the US as a potential biological control agent. This microsporidium infects natural larval populations of *Oc. japonicus* and *Oc. hatorii*. It invades larval fat body tissue and kills its host prior to pupation. The microsporidium is unikaryotic throughout development, undergoes asexual reproduction forming multinucleated schizonts and lanceolate spores in groups of 8 within a

sporophorous vesicle. Spores are orally infectious to mosquito larvae unlike other mosquito-parasitic microsporidia that require an intermediate copepod host. Orally infected larvae develop benign infections and survive to adulthood where the microsporidium is vertically transmitted by females. The resulting F1 progeny develop patent infections that lead to the production of infectious spores that are re-released into the aquatic environment with death of the larval host. Phylogenetic analysis of the small subunit rRNA gene sequence place this microsporidium as a distinct sister taxon within the clade of microsporidian parasites of mosquitoes. A new genus and species is proposed.

38 Benefits of aggregation to the mosquito parasitic nematodes *Strelkovimermis spiculatus*

Limin Dong, dlm666666@163.com, Yi Wang and Randy Gaugler

The aggregation behavior and its benefits for the mosquito parasitic nematode, *Strekovimermis speculatus*, have been studied in laboratory. The post parasitic nematodes aggregate into small mating balls and further merge into larger ones inside a sand plate (17 x 28 x 1.5 cm). The aggregation enhances the nematode mating rate and therefore increases reproductivity. Our data show that the mating balls also provide a male-male competition that may play an important role in the quality assurance of their offspring. The ability of a single male mating with multiple females could maximize the nematode reproduction when the population sex ratio is biased. The mating balls also assist in the nematode molting in unfavorable habitat such as a stagnant pond where perfect sized sand particles do not exist on the bottom. Molting is a critical process for the nematodes to turn into adults. The earlier molted nematodes have more opportunities to mate and reproduce. Forming a mating ball is a risk for their natural predators and therefore, the benefits must over weigh the risk for the behavior to exist.

39 Toxicological studies with larvae of *Aedes sollicitans and Aedes albopictus*

Lena B. Brattsten, brattsten@aesop.rutgers.edu, Debin Sun, Chansak Suwanchaichinda, Nga Q. Le and Lee D. Oliver

Aedes sollicitans and *Ae. albopictus*, although related species, have sharply differing life styles which may influence their relative responses to insecticides and other toxic compounds. We have studied not only traditional nerve poison-type insecticides but also compounds with other lethal modes of action and compounds not currently registered for use in larval mosquito control. We have investigated molecular genetic, biochemical, and biological characteristics of the 2 species. We were especially interested in the relative contributions to their responses to potentially toxic compounds by the defensive enzyme superfamilies, the carboxylesterases and the cytochrome P450s, with particular emphasis on the NADPH-cytochrome P450 oxidoreductase enzyme protein that is the common denominator for all cytochrome P450 activities. We will present recent and overview data from these studies that may be of value for guiding practical larval mosquito control strategies.

40 Evaluation of boric acid sugar baits against *Aedes albopictus* (Skuse) in residential communities in Florida

Diana P. Naranjo, dnaranjo@psy.miami.edu

Attractive toxic sugar bait (ATSB) application, using 1% boric acid, was evaluated against *Aedes albopictus* Skuse populations in the laboratory, semi-field trials, and field trials in residential communities in St. Augustine, FL. Laboratory evaluations of ATSB application to *Penta lanceolata* (Rubiaceae) demonstrated 100% and 92% mortality of *Ae. albopictus* at d 7 and 14, regardless of the flowers being left on or removed. A semi-field study evaluating ATSB application to the bottom or top portion of plants resulted in no significant difference on mortality ($P > 0.05$). Overall combined top and bottom ATSB application mortality at d 7 was 95% based on leaf bioassays. Field application of the ATSB-boric acid treatment did not decrease adult *Ae. albopictus* populations compared to the control site. However, oviposition was decreased significantly at d 14 ($P=0.001$) as monitored by oviposition traps. Attractive toxic sugar bait application in tropical environments still needs further evaluation for its efficacy, persistence and feasibility in controlling mosquito populations.

41 Preventing outbreaks of *Aedes aegypti*

Roberto Barrera, rbarrera@cdc.gov, Manuel Amador, Veronica Acevedo, Gilberto Felix, Belkis Caban and Andrew J. Mackay

Preventing dengue vector outbreaks is a research priority because it has been shown that dengue virus epidemics in San Juan, Puerto Rico, follow outbreaks of *Aedes aegypti*. We present the results of an investigation that suppressed outbreaks of *Ae. aegypti* using CDC autocidal gravid ovi-traps (AGO traps) in an urban community (325 buildings) that was compared with a reference community (280 buildings) without control traps. Females of *Ae. aegypti* were monitored weekly using BG Sentinel and AGO traps during pre- (October-December 2011) and post-intervention (December 2011- August 2012) periods. A generalized linear mixed model (GLMM) showed lack of significant differences in female mosquitoes in BG Sentinel and AGO traps between study sites during the pre-intervention period and highly significant effects of rainfall. A GLMM for all the data showed highly significant effects of the presence of the AGO control traps and highly significant effects of rainfall on the number of females mosquitoes in both BG Sentinel and AGO traps. The overall reduction of female *Ae. aegypti* in the intervention site was 60% after controlling for rainfall. The most salient effect of the AGO control traps was the suppression of *Ae. aegypti* outbreaks, which were caused by increased rainfall in the reference study site. Some advantages of the AGO control traps are that they do not use pesticides, are relatively inexpensive and durable, and do not require any maintenance for at least 2 mo.

42 A rapid colorimetric field test to determine residual activity of insecticides on surfaces
Daniel E. Szumlas, dszumlas@yahoo.com, Michael Green, Sylvester Coleman, Daniel Boakye, Maxwell Appawu and Samuel Dadzie

Indoor residual spraying (IRS) is one of the primary malaria prevention tools recommended by the World Health Organization (WHO). IRS insecticides employ any 1 of 15 insecticides from 4 insecticide classes, and longer lasting insecticides provide more protection to people living in endemic regions. WHO cone bioassays are used to measure mosquito mortality on insecticide treated walls, but such methods are often cumbersome in practice. Since mosquito bioactivity is a function of available insecticide on a treated surface, we developed and tested a simple and inexpensive colorimetric test to measure the amount of insecticide residue removed from surfaces. Our results show that colorimetric assays have strong potential to detect and measure insecticide residues on surfaces and have excellent potential to relate directly to results of WHO cone bioassays, the current gold standard for measuring insecticide bioactivity on treated surfaces. Applications for use in the USA are numerous and varied.

Disease Studies II

43 Effect of La Crosse virus infection on the production of neurotransmitters in vector mosquitoes
Fan Yang, yangfan@vt.edu, Carlyle C. Brewster and Sally L. Paulson

Adaptive evolution is a common phenomenon in which a pathogen can manipulate its vectors' behavior. Studies of arboviruses such as dengue virus and La Crosse virus (LACV) showed that virus infection of mosquitoes can cause an increase in virus transmission by altering the biting behavior of the vector. Several neurotransmitters, like serotonin and dopamine, have been found to play a role in controlling mosquito blood feeding behavior which include the host seeking, biting and feeding behavior. Previous work in our lab demonstrated that LACV-infected *Aedes triseriatus* took smaller blood meals and were twice as likely to feed multiple times than uninfected siblings. Our current data shows that serotonin, dopamine has been decreased in LACV-infected *Ae. triseriatus*. It will be really interesting to know whether the transovarially infected *Ae. triseriatus* will also alter their blood feeding behavior compared with uninfected mosquitoes. What kind of neurotransmitters in this transovarially infected *Ae. triseriatus* will change? If their blood feeding behavior has been changed and same neurotransmitter has been altered, we can conclude that LACV has this capability to change their vector blood feeding behavior and serotonin and dopamine, even their metabolites, involve in mosquitoes blood feeding behavior.

44 Potential for mosquitoes (Diptera: Culicidae) from Florida to transmit Rift Valley fever virus
Michael J. Turell, michael.turell@amedd.army.mil, Seth C. Britch, Robert L. Aldridge and Kenneth L. Linthicum

We evaluated 8 species of mosquitoes collected in Florida to determine which of these should be targeted for control should Rift Valley fever virus (RVFV) be detected in North America. Female mosquitoes that had fed on adult hamsters inoculated with RVFV were incubated for 7-21 d at 26°C, allowed to refeed on susceptible hamsters, and tested to determine infection, dissemination, and transmission rates. We also inoculated mosquitoes intrathoracically, held them for $\geq$7 d, and then allowed them to feed on a susceptible hamster to check for a salivary gland barrier. While *Culex nigripalpus* and *Anopheles crucians* were virtually incompetent, even when fed on hamsters with viremias $>10^{9.5}$ plaque-forming units (PFU)/ml, *Aedes atlanticus, Ae. infirmatus, Ae. vexans, Coquillettidia perturbans, Mansonia dyari,* and *Psorophora ferox* were all competent vectors of RVFV when fed on hamsters with viremias of $\leq 10^{8.3}$ PFU/ml. This represents the first time that either a *Mansonia* or a *Psorophora* species has ever been tested for RVFV, and *Ps. ferox* was the most efficient transmitter of the 8 species tested when exposed to a viremia $<10^{9}$ PFU/ml. In addition to laboratory vector competence, factors such as seasonal density, host feeding preference, longevity, and foraging behavior should be considered when determining the potential role that these species could play in RVFV transmission.

45 Altered response to attractive toxic sugar baits after infection of *Aedes albopictus* with Sindbis virus
Whitney Qualls, quallsamcd@bellsouth.net, John C. Beier, Gunter C. Muller, Rudy Xue and Doria F. Bowers

Altered response to attractive toxic sugar baits (ATSB) of adult female *Aedes albopictus* Skuse mosquitoes following dissemination of Sindbis virus (SINV; Family Togaviridae; genus *Alphavirus*) were observed at days 10 and 25 post-oral SINV bloodfeeding. Mortality of SINV exposed and control mosquitoes was evaluated against a 0.1%-, 1%-boric acid sugar bait and a non-toxic sugar bait at 24 and 48 h. There were significant differences in mortality of mosquitoes with a disseminated SINV infection at 24 h post-exposure to the 0.1% and 1% boric acid sugar bait at day 10 and 25 compared to control mosquitoes ($P < 0.05$). However, mortality was not significantly different ($P > 0.05$; 0.1% or 1% boric acid bait) at the 48-h evaluation period of SINV disseminated mosquitoes compared to control mosquitoes at day 10 and 25. Overall, we demonstrate that mortality occurs sooner in *Ae. albopictus* mosquitoes with a disseminated SINV infection. This data suggests that virus infected mosquitoes may be more susceptible to ATSB applications in the field.

46 Identification of canine heartworm and avian malaria in Tennessee mosquitoes
Rebecca T. Trout Fryxell, rfryxell@utk.edu, Hannah Peace, Taylor Thompson, Brian Hendricks, A. Daniel Greene, David Paulsen

Vector-borne diseases account for 22.8% of emerging infectious diseases and identifying the factors that influence disease transmission are critical especially with a constantly changing habitat (Jones et al. 2008). Two agents transmitted by a variety of mosquitoes include canine heartworm (*Dirofilaria* species) and avian malaria

(*Plasmodium* and *Haemoproteus*). Our objectives were to determine if canine heartworm and avian malaria are present in Tennessee field-collected mosquitoes and to identify the mosquito species that harbor each (or both) pathogens. During the summer and fall of 2012 mosquitoes were collected throughout Tennessee using CO_2 traps and gravid traps, and identified to species. Mosquito heads and thoraces underwent DNA extraction and parasite amplification via PCR for *Plasmodium* and *Haemoproteus* parasite DNA (cyt *b*) and for *Dirofilaria* species DNA (ITS-2 rDNA). All positive PCR amplicons were bi-directionally sequenced to confirm positivity and to identify the parasite genotype. The confirmation of avian malaria and canine heartworm in the sites and the identification of potential vector populations will begin to unravel the complex relationships that intimately tie together hosts, vectors, and parasites in the transmission of both pathogens. Results provide a significant contribution to the knowledge of the diversity of mosquito parasites present in Tennessee.

47 First detection of Heartland virus (Bunyaviridae) from field collected arthropods

Harry M. Savage, hms1@cdc.gov and William Nicholson

Ticks were collected at 3 sites at 2 farms owned by case-patients that became ill after infection with Heartland virus in 2009 (McMullan et al. 2012), at 3 sites on 2 farms not associated with human disease, at 6 sites located on 5 Missouri state conservation areas, and on the campus of Missouri Western State University. Tick collections occurred over 3 time periods in 2012: April 16-20, June 18-22, and August 6-10. Nine pools of *Amblyomma americanum* (L.) were RT-PCR positive for Heartland virus using 2 primer sets. All RT-PCR positive pools were composed of nymphs collected at farm 1. Eight pools were collected in April and one in June. The 8 pools from April collections yielded viable virus in plaque assays. The minimum infection rate (MIR) in nymphs from site 1 was 2.1 per thousand, 3.76 for April collections and 0.47 for June collections.

48 Climate change and mosquitoes: more or less?

Pat Dale, p.dale@griffith.edu.au, Jon Knight and Leila Eslami-Andergoli

The presentation explores the potential impact of climate and related changes on mosquitoes and suggests some key issues for management agencies to consider. Climate change will include temperature, rainfall and extreme events as well as associated sea level changes. Changing temperature will affect the development rates of mosquitoes and the pathogens they transmit. It will also influence the length of the main breeding season and spatial range of mosquitoes. The nature of the impact will vary with species and region. Rainfall changes will also affect mosquito habitats. For example it may affect the relative extent of mangroves and saltmarshes in tropical and sub-tropical intertidal areas, and hence the mosquito habitats. Locally it will directly affect patterns of mosquito production. The frequency of extreme events are expected to increase and this has implications for emergency response readiness for mosquito control. In addition, sea level is predicted to rise and this has impacts especially on coastal habitats. These issues are illustrated from the literature, with reference to a variety of examples. Operational mosquito management occurs at the local level and there is need to plan for contingencies over time scales relevant to the planning horizon of mosquito control agencies. As mosquito problems are generally associated with human settlement there also needs to be effective liaison between mosquito management agencies and urban and regional planning bodies.

49 Effect of climatic conditions on population dynamics of dengue mosquitoes (*Aedes aegypti* and *Aedes albopictus*)

Shabab Nasir, flourenceshabab@yahoo.com

This study was carried out to learn the effect of changing climatic conditions on population dynamics of *Aedes* mosquitoes. Mosquitoes were collected from fresh & sewage water ponds, fish ponds, rice fields, rain gutters, tires, tree holes, small water containers (drums, tanks, buckets) in and around the homes. Collection was done twice during every winter (Oct.-Feb.), summer (March-June) and monsoon season (July-Sep.). Years, months of the year, seasons, temperature and relative humidity were important in *Aedes* population dynamics. High population (63%) was recorded during rainy season because of high temperature (28-36°C) and high relative humidity (75%) and very low or zero population was recorded from outside during winter because of low temperature (<10°C) and low relative humidity (<23%); then the adults shifted in the rooms where they survived almost all the winter. *Aedes albopictus* was mostly recorded from exposed shady medium of rural areas and showed highly significant (p=0.00) association for seasons, water temperature and relative humidity. *Aedes aegypti* was largely recorded from tires, urban areas mostly during rainy season from small water containers and showed highly significant association with zones (p=0.008) and water temperature (0.038). Flowing and standing water also had significant effect on the population of *Aedes* mosquitoes. From this study, it was concluded that the areas with high rain fall had more oviposition sites and hence more mosquito population.

Legislative & Regulatory Symposium II

50 Mosquito control on federal lands: lingering matters and new wrinkles

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

The status for the USFWS completing and publishing for another round of public review-and-comment a second draft of their long awaited "National Mosquito Management Policy for National Wildlife Refuges" will be reviewed. The current word (October, 2012) from the USFWS is that their timeline for completing and publishing a second draft has now seemingly become "indeterminate," as opposed to their intention back in September, 2011 to have this done by winter or spring of 2012. The Service says they're now "rethinking some things" while also continuing to try to

complete their second draft, and that they'll let us know as soon as their new timeline might become more definitive. The AMCA stands ready to review and critique the second draft when available in our pursuit of a final "National Policy" that it is hoped will someday be acceptable to all pertinent parties. Several issues stemming from refuge-specific draft Comprehensive Conservation Plans (CCPs) or draft Mosquito Management Plans (MMPs) will also be reviewed, dealing with problems that have recently arisen regarding proposed mosquito control protocols or practices on-refuge, in part as a result of the USFWS's lack of an acceptable final "National Policy" to help craft the contents of these CCPs or MMPs. Finally, the status of an AMCA initiative for a proposed Federal Lands Mosquito Control Accommodation Act (FLMCA Act) will be reviewed, which if enacted by Congress would be applicable to all types of federal lands.

51 Clean Water Act, NPDES, and mosquito control: where do we go from here?
David Brown, dabrown@sac-yolomvcd.com

This discussion will provide the membership an update on NPDES regulations and what the future holds relative to mosquito control activities.

52 Public health pesticides: update on reregistration process?
Karl Malamud-Roam, kmr@aesop.rutgers.edu

Several public health pesticides are coming up for review and reregistration. The data needed to secure registration and the challenges we face to collect it will be presented.

53 Washington Day 2013: dealing with a new post-election political landscape
William H. Meredith, William.Meredith@state.de.us

The preliminary program, schedule and logistics for the AMCA's upcoming annual "Washington Day" event (more formally known as our "Annual Washington Conference") will be reviewed, to occur this year in May of 2013 in Washington DC. We once again urge as many of our AMCA members to attend as possible. Mention will be made of the most probable legislative or regulatory issues or needs that we'll try to tackle this year while in Washington, either during our visits to Senate and House offices on Capitol Hill, or via our interactions with key federal agency. Some indications of possible strategies or approaches relative to our priorities will also occur. The spring of 2013 could be a very exciting or challenging time for what we'll need to get done in Washington relative to our concerns and needs, and in our reacting to what'll probably be (for better or worse) a new post-election political landscape in D.C.

Latin American Symposium I

54 The importance of vacant lots in the production of *Aedes aegypti* mosquitoes in Merida, Yucatan, Mexico
Julián E. García-Rejón, julian.garcia.rejon@gmail.com, Carlos M. Baak-Baak, Roger Arana-Guardia, Mildred P. López-Uribe, Maria Puc-Tinal, Genny M. López-Uribe, Carlos Coba-Tún, María Alba Loroño-Pino, Carlos Machain-Williams, Guadalupe C. Reyes-Solis, Barry J. Beaty and Lars Eisen

Research conducted in Merida, Yucatan, Mexico, during the dry season investigated potential immature larval containers for *Aedes aegypti*. There were 544 sites including houses, cemeteries, streets, vacant lots and parking lots. A total of 2,309 sites were identified as potential oviposition sites for immature mosquitoes. A total of 39,680 immature mosquitoes were collected. The most abundant species was *Ae. aegypti* (31,929), *Culex quinquefasciatus* (6,220), *Cx. thriambus* (980) and others (551). Most of the immature *Ae. aegypti* were collected from vacant lots (15,962) and houses (7,222). Analyses of abundance of *Ae. aegypti* by type of container showed significant differences in the production of immature stages by type of container ($P = 0.005$). The highest numbers of larvae and pupae came from tires (13,772) and flowerpots (5,891), which together accounted for 49% of the total collection of *Ae. aegypti*. Tires and disposable containers produced the highest percentages of pupae (40% and 25%, respectively). Statistical differences between the presence of *Ae. aegypti* and the container's ability to maintain water were also observed ($t = -5.247$, $df = 168$, $P = 0.000$), most of the immatures coming from larger capacity containers (> 5 L of water). Higher densities of immatures were found in flowerpots and disposable containers.

55 Availability of controllable and disposable containers as habitat for *Aedes aegypti* in Huixtla, Chiapas, southern Mexico
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The availability of controllable and disposable containers as habitats for *Aedes aegypti* was evaluated in Huixtla, Chiapas City, between October and November 2009 and January and February 2010. We found a total of 30,180 containers in 250 houses inspected. Only 12.44% (3,755 of 30,180) containers were found to contain water and only 6.36% (239) were potential habitats for immature *Ae. aegypti*. A total of 682 pupae were collected, and of these, 91% (622) and 9% (60) were collected from controllable and disposable containers, respectively. Our results suggest that in Huixtla, Chiapas, controllable containers represent the most important container habitat for *Ae. aegypti*, and, of these, cement tanks were the most important containers producing mosquitoes during the wet and dry seasons.

56 Unusual oviposition sites of *Aedes aegypti* in Monterrey, Nuevo Leon, Mexico

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Aedes aegypti is considered as a domestic species and its oviposition sites are usually artificial containers. This study was carried out in order to find unusual oviposition sites for this species. Two areas in downtown Monterrey were selected for study. The first was a commercial and office area of 12 blocks and the second was an artificial river of 2.5 km length in a tourist area called "Paseo Santa Lucia." Entomological surveillance was carried out in both places. In the commercial and office area, *Ae. aegypti* was found in drainage containers and ornamental water spring, while in Santa Lucia oviposition sites were found in semipermanent wells, water spring breathers and in cavities of lamp. Larval *Anopheles pseudopunctipennis* and *Culex quinquefasciatus* were also found.

57 The CDC autocidal gravid ovitrap: a new surveillance tool for *Aedes aegypti*

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We describe the use of the CDC autocidal gravid ovitrap (AGO trap) to monitor gravid female *Aedes aegypti* and compare it with modified BG traps. The AGO trap uses hay infusion as a lure for gravid females that are captured on a sticky surface. The modified BG trap has a black exterior and does not contain the BG lure. The study was conducted from October 2011 to August 2012 in 2 urban communities (La Margarita, Villodas) in Puerto Rico. We conducted a control intervention in La Margarita using AGO traps and Villodas was observed as a control. Mosquito density was monitored with 42 AGO and 41 BG traps in La Margarita (325 buildings) and with 26 AGO and 26 BG traps in Villodas (280 buildings). BG traps were sampled for 3 consecutive days every week and AGO traps were sampled once a week. The number of females captured per AGO trap per day were 0.2 ± 0.01 (mean, SE) and 0.4 ± 0.02 in La Margarita and Villodas, respectively. Captures in BG traps per day were 0.67 ± 0.02 and 1.14 ± 0.03 in La Margarita and Villodas, respectively. Captures in both traps were significantly correlated ($r= 0.73$; $P< 0.01$). Relative sensitivity (percentage of positive traps) of AGO and BG traps per week was also significantly correlated ($r= 0.71$; $P< 0.01$). These high correlations indicate that AGO traps can be used to investigate the population dynamics of *Ae. aegypti*. We discuss the advantages and disadvantages of each trap for vector surveillance purposes.

58 Control of *Aedes aegypti* in storm sewers in Merida Yucatan with Natular™ DT and EC

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We report preliminary findings of ongoing field trials to determine the efficacy of 2 formulations of the larvicide Natular™ DT (multiple-brood tablet for containerized water) and EC (single-brood liquid) on the inhibition of the development of *Aedes aegypti* in 2 neighborhoods with 2 different types of storm sewers during the dry and rainy seasons 2012. Grated catch basins with a vertical drain pipe connected to a well were treated with Natular™ DT (one 1.35 g/tablet) and large dry wells connected to a side inlet with a concrete lid were treated with Natular™ EC (1 ml/m^2). Positivity (presence of 4^{th} instar larvae or pupae) for *Ae. aegypti* was measured by larval-funnel traps and exhaustive visual inspection at 48 h, and followed every week in all storm sewers found within 1 ha. Both formulations were highly effective larvicides against *Ae. aegypti*. Positivity in the treated sewers was reduced by 100% after the application. The duration of complete absence of larvae and pupae was confirmed after 8 wk during the dry season. During the rainy season, it was confirmed after 4 wk and assessment is still currently ongoing to evaluate persistence and residual effect with the natural water replacement and flooding. The selection of the type of insecticide used on street drainages in this area should be cautiously determined since it is directly discharged into the aquifer and potential contamination after continuous exposure can be detrimental to the subterranean water. Natular™ is an environmentally safe alternative and also an option for the management of insecticide resistance for local *Ae. aegypti* populations.

59 Effectiveness of temephos to control *Aedes aegypti* larvae using four application methods and two water conditions

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Temephos has been used for a long time to control *Aedes aegypti* larvae in Mexico. Its residual effect in plastic drums was evaluated with 4 application methods and 2 water conditions (*i.e.,* movement or not). Three levels of water were established in plastic drums; 20 g was the concentration used in this study and it was applied freely, in plastic bags, mesh bags and in a PET (polyethylene terephthalate) bottle; with and without water movement. Every week, 3 liters of water with the larvicide were taken from each level, application method and water condition. In the laboratory, 200 ml were placed in plastic containers and 20 3^{rd} instar larvae were exposed for 24 h, after which mortality was registered. Results suggested a small variation in mortality during 2 months.

60 Identification of dengue virus in natural populations of *Aedes aegypti* from Colombia during interepidemic periods

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Dengue is the most prevalent arbovirosis in Colombia, due mainly to the extensive distribution of its mosquito vector, *Aedes aegypti*. With the increasing number of confirmed cases, epidemiological monitoring is a priority. As part of this, virological surveillance in human and mosquito populations in endemic areas contributes to the

prevention and control of dengue outbreaks, reducing the risk of new dengue virus (DENV) infections. One of the prevention strategies includes early detection of dengue cases with the identification of DENV serotype 1 to 4 (DENV-1 to DENV-4), plus entomological surveillance. Considering that the introduction of new serotypes in human susceptible populations is one of the biggest risk factors associated with the emergence of dengue epidemics, we carried out virological surveillance of DENV in field-caught *Ae. aegypti* mosquitoes during 2009-2010 in urban areas at the municipality of Bello (Antioquia State), which reports co- circulation at least of 2 viral serotypes and confirmed cases of dengue hemorrhagic fever. The collections yielded 1,182 larvae (96%) and 52 adults (4%). Pools containing females, randomly chosen, were submitted to the RT-PCR/nested-PCR using specific primers for the NS3 region of DENV. The tests revealed the presence of DENV-3 in 2 female mosquitoes. Based on the results obtained, there was a minimum infection rate of 1.62/1,000 for *Ae. aegypti*. This study recorded evidence of the maintenance of the DENV in nature during interepidemic periods. Our results showed specificity for DENV of the NS3 region and good sensitivity to detect 0.2ng/ul of DENV. Virological surveillance of mosquito populations is a useful tool in the early warning systems and should be included, if possible, in epidemiological surveillance.

61 Genetic markers of virulence identified using full-length dengue viral genomes of isolates from epidemics in 21 Mexican states in 2011

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Dengue fever is caused by 1 of 4 dengue (DEN) viruses which belong to the family Flavivirus and are transmitted to humans primarily by the peridomestic mosquito, *Aedes aegypti*. The disease manifestations range from acute febrile DEN fever to DEN hemorrhagic fever or DEN shock syndrome. Recently, studies using regions of full-length genome sequences have shown the importance of specific DEN viral serotypes or genotypes in causing severe epidemics. Comparative analyses of complete viral genomic sequences from samples with well-defined epidemiologic and clinical records represent an alternative to define genetic markers associated with disease severity. In 2011, we sequenced the full length genome in collaboration with Dr. Irene Bosch (Broad Institute of MIT, USA) of 49 isolates obtained from patients from 21 Mexican states. We are searching for conserved genetic determinants associated with the DEN viruses' epidemiological severity and to mouse neurovirulence. Sequence analysis by bioinformatic tools is now underway to compare specific amino acid and nucleotide variations related with the dengue epidemiology. Results will demonstrate the interaction of genetic viral markers, the epidemiology of the disease, and protein structural modifications that contribute to the infection severity. This pioneering study is using full-length genome sequencing of the 4 DEN virus serotypes and genotypes from different geographic areas of Mexico. Moreover, these results will display the picture of DEN virus genetic diversity.

62 Microepi-entomology of dengue in a community in southern Mexico: ecological house of dengue

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Currently, there is no effective method for control of dengue transmission and there is no available dengue vaccine. Therefore, it is necessary to better understand the transmission of dengue based on its biological basis. We conducted a case-control study among households with cases of dengue hemorrhagic fever (DHF) and dengue fever (DF) and: 1) characterized variables of vector and host environments with significant association in high-transmission sites in Chetumal, Quintana Roo, Mexico; 2) quantified environmental-ecological factors that favor domestic densities of *Aedes aegypti* (longevity, resting sites, farms, demographics) and dynamics of vector densities; 3) determined places frequented by cases and controls (schools, factories, businesses; 4) determined dengue virus-infected mosquitoes (RT-PCR in households); and 5) identified the prevalence of previous infections in families whose houses were studied. In summary, it is an ecosystem study of microepi-entomology, which seeks to establish ecological circumstances between vectors, virus in the population most at risk, and compared in a case-control study (using DHF and DF cases as controls). We look for the existence of "aedic houses."

63 Risk of dengue reinfection associated to re-infesting *Aedes aegypti* populations in same home environments of past human cases in Monterrey, Mexico

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Dengue infections have expanded to other geographic areas such as the Americas as a result of changes in human ecology and behavior. Secondary dengue virus infections are a major risk factor for developing dengue hemorrhagic fever (DHF). Therefore, more recent exposure bites of dengue virus-infected *Aedes aegypti* females in previously diagnosed dengue cases fulfills a component of the epidemiological model of DHF. A study population of 357 dengue fever (DF) and 43 DHF cases which were confirmed by laboratory tests and clinical manifestations was selected. A cross-sectional entomological survey was conducted inside of their homes and in their backyards. Pooled numbers of houses where old DF and DHF cases were living showed 268 (67.0%) with wet and dry *Ae. aegypti* oviposition sites present. One hundred thirty-two (33%) householders with past DF and DHF infections showed good knowledge of either larval or adult dengue vector mosquitoes. Pooled data of DF and DHF cases showed 28.4% of households with larval habitats, while adult *Ae. aegypti* were found in 8.0% of the houses. According to gender distribution, studied households represented 145 (36.2%) DF and 14 (3.5%) DHF with men confirmed as dengue cases. On the other hand, DF and DHF houses included 212 (53%) and 29 (7.3%) women. There is an entomological factor playing a role in DHF epidemiology. Secondary dengue infections can be caused when those who have suffered the disease in the past become exposed, and ignore, partially controlled vector populations.

64 The potential of non-residential sites in the dengue virus transmission in Monterrey, northeastern Mexico
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Dengue is one of the major public health problems in Mexico and Latin America. Year after year there are outbreaks of dengue despite existing vector control programs. One reason that favors outbreaks is that the control of *Aedes aegypti* is often only performed in houses while non-residential sites (e.g., schools, churches, factories) are often ignored. This study was aimed at assessing the potential epidemiological role of dengue vector densities prevailing in private and public spaces. A total of 86 non-residential sites were sampled for the presence of potential and active oviposition sites and adult mosquitoes. The female *Ae. aegypti* that were collected were tested for dengue virus (DENV) RNA. *Aedes aegypti* oviposition was recorded at 80.2% of the sampled sites, whereas adult *Ae. aegypti* were present at 94.2% of the sites surveyed. Schools and recreational sites had higher mean numbers of female *Ae. aegypti* (21.6 and 10.4, respectively). The Chi-square test showed no significant differences in the presence of the dengue vector at selected sample site categories. Indoor use patterns of resting adult mosquitoes indicated that the bathroom and classrooms were the preferred resting sites. As for the type of oviposition site, the smaller ones were the most abundant (62.8%). We did not collect any female *Ae. aegypti* with DENV. Importantly, daytime human activities (e.g., attending school or working) is synchronized with the bimodal biting rhythm of *Ae. aegypti*, increasing the chance of DENV transmission.

65 Vertical transmission of dengue virus in *Aedes aegypti* associated with villages with historically intense dengue transmission in southern Mexico
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An ecological study was designed to identify epidemiological patterns inside of dengue transmission networks at the village level in 4 states (Veracruz, Oaxaca, Morelos and Chiapas) in southern Mexico. We explored the potential vertical transmission of dengue virus in *Aedes aegypti* in 10 villages in these states where historically intense dengue outbreaks occurred between 2001 and 2011. The study was conducted in areas when cases of dengue fever and hemorrhagic dengue were recorded by the ministry of health's epidemiological surveillance system. In each area, a buffer of 200 m was established and 30 houses were selected at random. A total of 60 plastic cans (105 mm high and 772 mm diameter) were painted black and were used as ovitraps inside and outside the houses. The number of eggs collected in each trap was recorded and eggs were transported to the insectary to allow them to hatch and develop into adults. The presence of dengue virus in the field-collected specimens (pool size of 30 females and males) was detected by reverse transcription-polymerase chain reaction (RT-PCR) assays, followed by sequencing, and by detection of infected mosquito C6/36 cells using indirect immunofluorescence with polyclonal antibodies. We found evidence of vertical transmission in *Aedes aegypti* in villages in the states of Chiapas (Tapachula), Veracruz (Veracruz Harbor and Coatzacoalcos), Morelos (Axochiapan, Quebrantadero), and Oaxaca (Puerto Escondido, Santa Maria Huatulco) with DEN-1, DEN-2 and DEN-3 serotypes. Maps of occurrence were produced by GARP approach to model the ecologic niche in order to identify some other areas where potential vertical transmission of dengue virus could exist in *Aedes aegypti*.

Public Relations Symposium I

66 Introduction to the symposium
Joseph Conlon, conlonamcata@gmail.com

The intense media coverage brought about by the extraordinary 2012 West Nile virus and eastern equine encephalitis outbreaks renewed the public's interest in mosquito control strategies undertaken to curtail these epidemics. Broadcast media, in particular, sought to shed light on both the efficacy and risks involved in mosquito-borne disease control measures brought to bear in both the Eastern Seaboard and the Great Plains. A great deal of misinformation was conveyed through these outlets by various "experts", whose knowledge of proven mosquito control operations was seriously flawed and, if unchallenged, would lead to greater risk for the populace we are charged to protect. This symposium will detail the public relations challenges facing vector control organizations; define our message to the public health stakeholders; detail the science delineating the risk to the populace of vector-borne disease as compared to mosquito control operations; and provide on-site training regarding properly conveying this risk message.

See the History, Be the Future

67 No simple solutions: a century of organized mosquito control
Gordon Patterson, patterso@fit.edu

At the beginning of the twentieth century, a handful of physicians, entomologists, engineers, and publicly minded individuals began the campaign for organized mosquito control in New Jersey. In 1912, Governor Woodrow Wilson signed into law a bill authorizing the formation of the nation's first mosquito control districts. During the next hundred years, New Jersey served as a catalyst and model for the development of a nation-wide anti-mosquito movement. This paper will present a brief summary of some of the New Jersey's most notable accomplishments in guarding the public's welfare from the mosquito menace.

68 The beginnings of county commissions in New Jersey: Essex and Union County histories
Eric Williges, ewilliges@essexcountynj.org, Ralph Strano and Sean Devaney

Organized mosquito control began in New Jersey with John B. Smith's efforts to develop methods to survey and control mosquitoes across the state. His efforts culminated in the adoption of the 1912 law directing the statewide creation of county mosquito extermination commissions. The first commissions empowered under this law were the Essex County Mosquito Extermination Commission and the Union County Mosquito Extermination Commission, both created in 1912. We examine the early history of these 2 organizations and how they began their ongoing attempts to control mosquitoes within their borders.

69 Arboviral disease outbreaks in New Jersey: then and now
Tina Tan, shereen.semple@doh.state.nj.us

Human disease associated with arboviral infection was first identified in New Jersey (NJ) in 1959, when 32 residents were diagnosed with eastern equine encephalitis (EEE). Over the course of one summer, 22 of those individuals died and many others were left with long-term neurologic deficits. In 1964 and 1975, the state experienced outbreaks of St. Louis encephalitis (SLE); over 100 residents were sickened, and 9 died from encephalitis caused by the mosquito-borne virus. In response to these outbreaks, public health, mosquito control and veterinary professionals partnered to initiate cooperative surveillance and prevention campaigns in NJ. With the emergence of West Nile virus (WNV) in the United States in 1999, NJ re-established its public health and mosquito control partnerships and created an interagency Vector-borne Disease Working Group (VBDWG). The group meets monthly to discuss arboviral disease planning and control, with an emphasis on timely surveillance, communication, and interagency cooperation. With issues such as climate change and emerging vectors, and international travel becoming more frequent and commonplace among residents and visitors, NJ will continue to work in a coordinated and integrated fashion to prevent, detect and control outbreaks of emerging and re-emerging pathogens.

70 Novel New Jersey contributions to mosquito surveillance
Robert D. Duryea, bduryea@warrencountymosquito.org

Mosquito surveillance is an important aspect of mosquito control. Many unique surveillance devices and techniques have come out of NJ over the past 100 years. A brief overview of the resting box, Ehrenburg pigeon trap, truck mounted catch net, and bilge pump for *Coquillettidia perturbans* sampling are given.

71 Counting mosquitoes in New Jersey: past, present and future
Lisa Reed, lreed@rci.rutgers.edu

Being able to know how many mosquitoes are in an area is the first step toward control. This talk presents the history of the development of the New Jersey light trap at Rutgers University from Sugar Can to Model 50 and its use as a monitoring device of mosquito populations. Advantages include the attraction of significant pestiferous and public health mosquito species and the ability to calibrate for comparisons, a unique feature among traps. Disadvantages include difficulties of ease of use. Historical mosquito patterns in comparison to current patterns will be presented, along with potential future devices in the estimation of mosquito populations.

72 A synopsis of salt marsh water management including 35 years of applied open marsh water management in Ocean County, New Jersey
Richard Candeletti, rcmosquito@comcast.net, Joseph F. Schmidt, Michael G. Senyk and Thomas M. Candeletti

The development and application of open marsh water management (OMWM) as pioneered in New Jersey will be discussed. This will include a review of early attempts of salt marsh mosquito source reduction through hand ditching prior to the 1900s, as well as parallel ditching as conducted under CCC and WPA projects. Current OMWM techniques and equipment as used in Ocean County, NJ, will be presented along with control success and cost effectiveness data. In conclusion, the possible present and future refinements of OMWM will be addressed.

73 Have you ever seen a mosquito eating fish? A brief history of larvivorous fish in New Jersey
Anthony J. Acquaviva, tacquavi@co.monmouth.nj.us

At the start of the twentieth century, mosquito control workers began to evaluate the viability of the use of larvivorous fish to control mosquitoes in bodies of water in New Jersey. Through trials and tribulations, along with careful scientific research, a modern and efficient biological control program featuring predaceous fish was established. This program now has decades of success and is growing in importance with the housing crisis and the concomitant increase in derelict swimming pools. This presentation will highlight the noteworthy historical developments in turning a concept into a fully functional mode of mosquito control.

74 Mosquitocides: past, present and future
Greg M. Williams, gwilliams@hudsonregionalhealth.org and Tom Candeletti

New Jersey is well known for establishing organized mosquito control in this country. Less obvious is the role that New Jersey played in the development of many of the pesticides that made these programs possible. Since Joseph Ginsburg created the first mosquito adulticide at Rutgers University in the early 1930's, New Jersey has made significant contributions toward the advancement of mosquitocide technology. This talk will review many of the

most successful mosquito control products to come out of New Jersey as well as current and future work aimed at expanding the mosquito control arsenal.

75 The evolution of aerial operations in mosquito control work
Ralph Strano, rstrano@UCNJ.ORG

Mosquito control work began to organize in a science-based approach at the same time that the Wright Brothers were successful with their heavier-than-air craft. The future progress of both fields has proven to benefit the public here and around the world. This presentation will touch on the history of the 2 sciences and how they had an inter-relationship that exists to this day. The combination has been able to increase the food supply in the world, and for our purposes, has been able to accomplish mosquito control over vast areas that otherwise would prove to be virtually impossible.
In 1910, Glen Curtiss demonstrated the first capabilities of aircraft for destructive purposes during war. Not long after, mosquito control work was using aircraft to wage its own war against mosquitoes for peace and relief of nuisance and disease! A timeline of events and inventions will be presented here to illustrate the history of aerial pesticide applications. With the work of Sean Devaney from Union County Mosquito Control, Scott Crans from Rutgers Center for Vector Biology, and the Archives Committee of the New Jersey Mosquito Control Association, we present the "Evolution of Aerial Operations in Mosquito Control Work".

76 Mosquito education and outreach: why it pays to do your homework
Kristen Bartlett-Healy, krisbh@verizon.net

In 2013, the New Jersey Mosquito Control Association will be celebrating its 100-year anniversary. The past 100 years have shown dramatic changes in public education and outreach for mosquito control in New Jersey. A significant trend in the improvement of public education and outreach has been understanding the target audience, and building programs around their needs and education levels. This includes conducting surveys and focus groups, and evaluating the efficacy of programs through surveillance and feedback. Our education and outreach efforts in New Jersey have a strong focus on elementary school level, as well as more active adult programs.

Biorational Larvicides and Larviciding I: Resistance and Resistance Management

77 Resistance management: opportunities and challenges
Janet McAllister, jvm6@cdc.gov

There are 3 generally accepted theories of management for insecticide resistance: management by saturation, management by moderation and management by multiple attack. The best method for resistance management will vary depending on the situation in which control is to be accomplished and integrating elements of all 3 may be necessary to mitigate the problem. The 3 theories will be discussed in the context of larval control but also apply to adult control.

78 Susceptibility monitoring and resistance management: be proactive!
Maria Helena N. Silva-Filha, mhneves@cpqam.fiocruz.br, Karlos Diogo M. Chalegre, Tatiany A. Romao, Daniella A. Tavares, Eloina M. Santos, Maria Alice V. Melo-Santos, Cláudia F. Oliveira, Osvaldo P. de Melo-Neto and Lêda Regis

Bacillus sphaericus (*Bsp*) and *Bacillus thuringiensis* (*Bti*) based larvicides have been used in Recife city, Brazil, to control *Culex quinquefasciatus* and *Aedes aegypti*, which are vectors of filariasis and dengue, respectively, in that area. Approaches for monitoring susceptibility to these biolarvicides were developed. *Bsp* resistance among *Cx. quinquefasciatus* has been recorded worldwide and in this study aimed to characterize and screen resistance alleles. Four alleles of the *cqm1* gene were identified among Recife populations and those display mutations that prevent the expression of functional Cqm1 receptors for the binary toxin and, consequently, provoke resistance. The mutations are located in a hotspot of that gene, and this facilitates their detection by a single PCR method, and the evaluation of allele frequencies among populations has provided valuable data to access the risk of resistance. In the case of *Bti*, its complex mode of action does not favor the resistance selection; however, some reports suggested an association of temephos resistance with the decrease of *Bti* susceptibility. With this aim, *Ae. aegypti* populations were analyzed and they displayed similar susceptibility to *Bti*, regardless of the temephos resistance status and/or increased activity of detoxifying enzymes. Data show that *Bti* is suitable to overcome resistance to temephos and reinforce the lack of cross-resistance between these larvicides.

79 Toxin interactions and resistance management of *Bti* and *B. sphaericus*
Margaret C. Wirth, mcwirth@ucr.edu, William E. Walton and Brian A. Federici

Microbial insecticides, specifically *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *Lysinibacillus sphaericus*, formerly *Bacillus sphaericus* (*Bs*), are major components of many mosquito control programs, particularly programs targeting *Culex* species. Both are effective larvicidal agents but differ in their risk for the evolution of resistance. *Bti* has demonstrated a low risk for resistance when used for long-term treatment against mosquito larvae because the complex toxin mixture native to *Bti* and the associated toxin interactions therein, are critical to its refractoriness to resistance. Conversely, *Bs* carries a higher risk for resistance because of its single-site action; but resistance may be avoided if specific toxin interactions are exploited. Toxicology studies, toxin interaction studies, and selection studies have helped elucidate many of the characteristics that are key to reducing the long-term likelihood for resistance while increasing larvicidal activity and enhancing host range. Data from multiple studies will be presented to

illustrate the fundamental traits that are key to a highly active larvicidal product that is also refractory to resistance. Results of laboratory selection studies and field data will be shown to illustrate the various strategies that have been demonstrated to be effective for resistance management.

80 What happened after 30-year application of *Bti* formulations in Germany?
Norbert Becker, norbertfbecker@web.de

The use of *Bti* in Germany is a unique story of success. Our goal to protect our citizens against floodwater mosquitoes and the sensitive floodplain ecosystems has been realized with the use of *Bti*. The use of bacterial larvicides in our program comprised different phases: 1) 1st attempts with our own fermented *Bti* products in the late 1970s including selectivity tests and field efficacy studies; 2) early 1980s large scale application and development of *Bti*-SG based on commercial *Bti* products for aerial application; 3) intensive studies on the direct impact of *Bti*-treatments on the aquatic ecosystem have been conducted to demonstrate the safety of the methodology and to address environmental concerns; and 4) since 1983 the routine control is based only on the use of improved formulations and application systems. In 1992, *Bti*-tablets as well as gamma-radiation were developed for the use of *Bti* (AM65-52) in the fight against container-ovipositing mosquitoes. Since 2006, the ice granule technique has been implemented to allow aerial application in a cost-effective way. Since the beginning, about 20,000 tons of various *Bti* formulations have been used to treat more than 300,000 ha successfully without any sign of resistance or visible adverse effect on the ecosystem. Reduction of adult mosquito populations is always more than 95%, which is very much appreciated by the public.

81 Profile of resistance and cross-resistance to spinosad in *Culex quinquefasciatus* (Diptera: Culicidae)
Tianyun S. Su, tsu@wvmvcd.org and Min Lee Cheng

Laboratory studies were carried out to explore the potential of resistance development in *Culex quinquefasciatus* to spinosad and cross-resistance to other commonly used mosquito larvicides. Low to moderate levels of resistance to spinosad was established in response to intense selection pressure. Generally, cross-resistance to other microbial, IGR and organophosphate larvicides did not exist in a spinosad-resistant population, except to *Bacillus sphaericus* where cross-resistance levels to this microbial agent were even higher than resistance to spinosad. However, *B. sphaericus*-resistant population did not show any cross-resistance to spinosad. Resistance management strategies were discussed.

Latin American Symposium II

82 Malaria vector species in three endemic regions of Colombia: distribution and human-vector contact
Martha L. Quinones, marthalquinones@gmail.com, Martha L. Ahumada, John C. Beier and Socrates Herrera

About 83% of the malaria cases of Colombia occur in the western part of the country, mainly along the Pacific coast and in the departments (states) of Cordoba and Antioquia. The purpose of this study was to determine the distribution of the malaria vectors in those areas and their human-vector contact. Samples of *Anopheles* mosquitoes were collected in a total of 70 sites between May 2011 and August 2012 in 3 areas (Cordoba, Valle and Nariño), using human landing catches from 18:00 to 24:00 h, indoors and outdoors simultaneously, and in 8 houses for a week in each locality. Mosquitoes were identified using the existing morphological keys and, for some problematic specimens, sequences of COI were used to confirm the species. A total of 6,697 mosquitoes were collected. In Cordoba, 8 species were found and *An. nuneztovari* was the most abundant species (74%), followed by *An. darlingi* (13.1%) and *An. albimanus* (9.2%). In Valle, 7 species were found and *An. nuneztovari* had the highest human-vector contact (72.7%), followed by *An. albimanus* (23.9%), and *An. neivai* (1.7%). In Nariño, 5 species were collected and *An. albimanus* comprised 89.9% of the specimens collected, followed by *An. calderoni* (9.5%). The human landing rate was higher in the outdoors in all 3 areas, compared with the indoors (between 52% and 81.7%), for all species. This important proportion of outdoor human-vector contact, points out the need for complementary control activities together with long-lasting treated nets or indoor residual spraying.
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83 Vegetation land cover and malaria transmission in the lower Caura River Basin, Venezuela
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To explore the effect of deforestation and resulting different vegetation land covers on malaria transmission, 3 villages were selected in the Lower Caura River Basin, Sucre Municipality, Bolívar State, Venezuela. All night mosquito collections were conducted between March 2008 and January 2009 using CDC light traps and Mosquito Magnet® Liberty Plus. Human landing catches were carried out when anophelines were most active (between 1800 and 2200 h). A Landsat 7 ETM + image was interpreted and unsupervised classification was performed using module ISODATA in IDRISI TAIGA in combination with the module SEGMENTATION for supervised classification. The final vegetation and land cover classes were validated in the field. Four types of vegetation land cover were identified: primary forest, late secondary vegetation, early secondary vegetation, and bare soil-subsistence scale agriculture. The Annual Parasite Index was not related to the type of vegetation. *Anopheles darlingi* and *An. nuneztovari* were the most abundant species and were collected in all 3 villages. Jabillal, the least forested village had a higher anopheline abundance, biting rate and species diversity. The more forested village of El Palmar reported a higher entomological

inoculation rate. The results confirmed previous observations that the impact of deforestation and resulting vegetation cover on malaria transmission is complex and varies locally. Financial support: International Development Research Centre (IDRC – Canada) (Project-103696-006)

84 Spatial distribution of reemerging vector borne diseases (Chagas, malaria and dengue) in the state of Jalisco, Mexico.

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The objective of this study was to characterize the spatial distribution of several reemerging vector-borne diseases (e.g., Chagas, malaria and dengue) in the state of Jalisco, Mexico. We searched for the vectors of these 3 diseases in 10 municipalities; 10 households in each municipality were sampled. Each household was surveyed and a vector knowledge questionnaire was applied to residents. A backpack aspirator was used to collect mosquitoes in peridomiciliary and intradomiciliary areas. Mosquito larvae and triatomine bugs were sampled by an active search (1h man/housing). In addition, we placed an ovitrap both indoors and outdoors at each house which was checked after 10 days. The captured insects were placed in containers and labeled with the date of collection and transported to the laboratory where they were identified, counted and mounted to form part of the entomological reference collection. The mosquito *Aedes aegypti* was found in 6 municipalities while *Anopheles pseudopunctipenis* was only found in 1 municipality. As for the vectors of Chagas disease, we collected *Triatoma longipennis*, *T. barberi* and *T. picturata* in 6 municipalities.

85 The potential risk for the transmission of Chagas and leishmaniasis in northeastern Mexico

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Chagas and leishmaniasis are endemic diseases in Latin America. In Mexico, these illnesses are known to occur in several areas of the country, but especially in the central and southern regions. Usually, the health authorities do not consider these diseases as health problems in northern Mexico. However, recent findings gathered by our interinstitutional and interdisciplinary research group indicate that there is a present risk for the transmission of both parasites (*Trypanosoma cruzi* and *Leishmania exicana*). In the northeastern states of Coahuila, Nuevo León and Tamaulipas, we have found the following biological elements: (1) new geographical records of triatomine bugs (*Triatoma rubida* and *T. gerstaeckeri*) with infection data; (2) new geographical records of phlebotomine sand flies (12 species); (3) clinical and serological evidence of cases of Chagas disease; and (4) confirmation of infection of the host *Sigmodon hispidus* with *L. exicana*. This new evidence led us to suggest that these parasites are being maintained in enzootic cycles and that it may be possible that more human cases are currently undiagnosed. In the light of these findings, we suggest that more detailed studies are needed to confirm our hypothesis in order to unravel the full extent of the prevalence of infection in humans, vectors and reservoir hosts of these neglected diseases, and thereby assessing the overall risk of transmission to humans in the northeastern states of Mexico.

86 West Nile virus survey on birds, horses, and mosquitoes of the Pacific coast of southern Mexico

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West Nile virus (WNV) has caused hundreds of cases of encephalitis, myocarditis and breathing difficulties and is especially serious in the elderly. In 2002, serologic evidence of WNV infection was detected in horses, chickens and resident free-ranging birds in Guadeloupe, the Dominican Republic, and eastern Mexico. The objectives of this study were to update data in horses and birds with WNV antibodies by ELISA (enzyme-linked immuno sorbent assay) and attempt to detect this virus in mosquitoes by RT-PCR (reverse transcription polymerase chain reaction). Field work was conducted in villages in the municipalities of Ometepec, Acapulco and José Azueta to gather evidence of WNV circulation in this state (Guerrero on the southern Pacific Coast of Mexico). We captured 40 birds in mist nets from which blood samples were collected and analyzed by ELISAs. Also, 102 equine blood samples were collected and tested as the bird samples had been tested. In addition, 4,854 mosquitoes were caught using CDC light traps and motorized backpack aspirators. Of the 40 birds sampled, 4 (10%) tested positive for WNV antibodies while 19 (18%) of 102 horses were found with WNV antibodies. West Nile virus was not detected in any of the 116 mosquito pools tested by RT-PCR assay. On the coast of Guerrero state, WNV was found to be circulating between birds and horses but no evidence of WNV was found in mosquitoes.

87 Indoor entering and exiting behavior of *Lutzomyia longiflocosa* in rural houses in Colombia

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This study identified the entering and exiting sites for *Lutzomyia longiflocosa* in rural houses. Two types of sites were tested: a) big open area around eaves and walls and b) small cracks on doors and windows. Entering sites were identified by collecting the sand flies caught on sticky traps set up outside the bedroom around the big open area and in large cages fitted on doors and windows with small cracks inside the bedroom. Exiting sites were identified by releasing groups of wild caught *L. longiflocosa* females indoors, previously marked and blood-fed. Females were recaptured using the methods described above but set up in the opposite position. In the entering experiment, most (98.8%) of all sand flies collected were caught near the big open area. A higher percentage (72.9%) and abundance of sand flies were caught on the front side compared with the back of the house, 9.8 (95% C.I.= 6.1 – 15.5) vs. 1.0 (0.3 –

2.1) sand flies/m^2, respectively. In the exiting experiment most of the recaptured females were caught near the big open area (90.8%) and on the front side of the house (63%). These results suggest that *L. longiflocosa* enter and exit houses mainly through a large open area on the front side of the house.

88 Mosquitoes (Diptera: Culicidae) and their importance in the public and animal health in Chetumal, Quintana Roo, southern México

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A checklist of mosquito fauna found in the Chetumal area of Quintana Roo state in southern Mexico is presented. We used data of previously identified species from the available bibliography along with species field surveys made by the authors since 2010. A total of 5 genera and 16 species are described. Diagnostic considerations and public health and veterinary importance of some culicids are given. High mosquito densities were observed with the Shannon index during May and September. Species abundance was analyzed using contingency tables between types of oviposition sites and the number of species captured by month. The richest genera were represented by the genera *Aedes* and *Culex* followed by *Wyeomyia*. *Aedes aegypti* was the most abundant species collected during all sampling months followed by *Culex quinquefasciatus*. Of all the species collected in the urban area of Chetumal, only 3 species were not associated in disease transmission, while all other species were incriminated in outbreaks of vector-borne transmission of parasites or arboviruses.

89 Surveillance and management challenges of *Aedes albopictus* in Mexico

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In 1985, *Aedes albopictus* was introduced into the United States through the port of Houston, TX. Four years later, this species was identified in Matamoros, Mexico, a bordering location about 400 mi from Houston. Since that time, *Ae. albopictus* has dispersed throughout the country reaching southern borders with Guatemala (Tapachula) and Belize. Entomological surveillance started during the 1990s and concentrated on *Ae. albopictus* movement mostly in 3 northern states (i.e., Tamaulipas, Nuevo Leon and Coahuila) of Mexico. Unlike the United States, arrival of *Ae. albopictus* into Mexico presented the country with endemic dengue epidemics. In 1995, *Ae. albopictus* was found with dengue virus serotypes 2 and 3 infections in Reynosa, another south Texas border city. Dengue virus was isolated from a pool of male mosquitoes indicating vertical transmission. During 2011, a second report of *Ae. albopictus* vertically-infected with dengue virus was found near the city of Monterrey. There, populations of *Ae. aegypti* and *Ae. albopictus* share rural and suburban areas, and the circulation of dengue viruses is prevalent annually. The epidemiological role of *Ae. albopictus* in Mexico is becoming worrisome for Mexican health programs. The West Nile virus outbreak in Dallas, TX, may trigger the vectorial competence of *Ae. albopictus* in northern Mexico to become infected with this arbovirus.

90 Molecular relationship between members of *Anopheles albitarsis* complex from Colombia

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According to mtDNA and nDNA analyses in Colombia there are several members of *Anopheles albitarsis* complex. Some of these have been implicated as malaria vectors, particularly in the Orinoquia Region. We analyzed members of this group that were collected in several localities of Colombia to determine how many lineages we could find and to understand the relationships between them. Using nDNA, we use *white* gene to separate individuals who do not retain intron IV (associated to *An. albitarsis* F) from others who retain intron IV associated with other species. For the mitochondrial analysis, we used barcode region from COI subunit gene. Sequences were aligned, eye checked and edited. Trees were built using the Tamura-Nei distance model and Neighbor-Joining algorithm, with 1,000 replicates to obtain a consensus tree. The comparison between trees agreed with the evolutionary rate of each marker. *White* gene produce well defined clusters into the tree, meanwhile mtDNA did not. In Colombia, the existence of several *Anopheles albitarsis* complex members helps contribute with more entangled malaria scenarios.

91 Recycling of nematodes, *Romanomermis culicivorax*, in larval sites of *Culex quinquefasciatus*

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The establishment of postparasitic *Romanomermis culicivorax* nematodes in larval sites of mosquitoes is an alternative application method to guarantee long-term control of larval mosquito populations. Four sites with *Culex quinquefasciatus* larvae were established with a substrate of 10 cm of gravel (pebbles) as well as 2 additional larval sites with a substrate of soil. Two hundred postparasitic nematodes (100 males and 100 females) were applied to the 2 gravel sites and in the other 4 sites (2 with gravel and 2 with soil), 100 nematodes (50 males and 50 females) were added. Nineteen d after the addition of the nematodes, 500 mosquito larvae were added to each larval site as well as 20 larvae in a sentinel trap to assess the potential activity of the nematodes. After 5 d, the trap was recovered and the mosquito larvae were dissected to determine prevalence and intensity of infection. During the first 100 d, mosquito larvae were added to the sites weekly in sentinel traps. After 100 d, the mosquitoes were added and evaluated every 2 wk and after 250 d the interval was increased to 3 wk. The recycling study was continued for 468 d. Infection prevalence of 30 to 45% were observed. The major intensity of infection was observed in larval sites with a gravel substrate.

Biology/Behavior

92 *Aedes albopictus* resting and sugar feeding behavior in vegetation commonly found in residential backyards in St. Augustine, FL

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The aim of this study was to further investigate the resting and sugar feeding behavior of *Aedes albopictus* Skuse by aspirating resting mosquitoes in and around common landscaping vegetation found in residential communities in St. Augustine, FL. We identified 6 plants, some flowering and non-flowering, and 9 samples were taken from the selected sites and vegetation throughout several weeks in June. Eight mosquito species were collected with *Ae. albopictus* representing 74% of the overall collection. The number of *Ae. albopictus* collected varied significantly with the type of vegetation ($F=25.3, P < 0.05$). When comparing the abundance of the mosquitoes on the different vegetation, the highest percentage of *Ae. albopictus* were collected resting in the Mexican petunia followed by fern and Tahitian bridal veil and then by plumbago, purple heart, and hibiscus. Also, resting *Ae. albopictus* collected in Mexican petunia had significantly more lipid reserves ($F=3.45$, $P=0.014$) compared to the other plants sampled. These findings highlight the potential correlation between sugar feeding and resting behavior. The results of this study can increase more efficient monitoring and surveillance by focusing on certain plants. The preference of mosquitoes for certain vegetation will help target control strategies.

93 Loss of bet-hedging in ovipositing female *Aedes albopictus*, Skuse, in the fall

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The Preference-Performance hypothesis posits that females oviposit in sites that maximize the future performance of the offspring. Consequently, they may be forced to choose a "sub-optimal" habitat when "optimal" sites are unavailable, increasing the diversity of habitats used. Developed for herbivorous insects, this hypothesis has implications for mosquito surveillance. We tested the effect of the presence of conspecific eggs on the oviposition preference of female *Aedes albopictus* under early summer (L:D: 16:8 h) or fall (L:D: 10:14 h) light regimes. Under controlled laboratory conditions we gave gravid females a choice of containers with leaf infusion (=food) or water (=no food) either open or with a single small (3 x 5 mm^2) opening (crossed design, 4 habitat treatments per cage). We found that open containers with leaf infusion are the preferred habitat (=are the first ones accumulating eggs), but also that summer eggs will accumulate in "sub-optimal" conditions until no difference can be found between container types. In contrast, eggs laid by females that have experienced low light levels (=diapausing eggs) accumulate mostly in the preferred habitats. Further experiments revealed that females laying diapausing eggs preferred habitats with conspecific eggs.

94 Larval coloration as an anti-predator defense in *Culex pipiens*

Lydia Wang, secrlickq@gmail.com, Linda Dong, Yi Wang and Randy Gaugler

Culex pipiens pipiens, one of the most successful and widely distributed mosquito species, is a vector of West Nile virus and other diseases. Colored larvae of this species have been observed in lab culture and field collection. The colors are maintained through pupae and adults. The colors, including blue, purple, green and orange, are the result of a recessive mutation. Our question was whether the color mutation is neutral or provides a selective advantages. Our field data indicated that higher number of some colors was associated with container or habitat color. For example, higher numbers of purple or blue larvae were observed in blue containers, whereas more green larvae were collected from green habitats. Several species of mosquito predators were collected from the larval habitat and tested for selection pressure against different colored larvae against different backgrounds. The results indicated that colored mosquito larvae survived better in a habitat with similar color. We conclude that color mutations impart a selective advantage to the larvae through cryptic coloration. The strategy of survival and fitness related to the color mutation is also discussed.

95 Molecular identification of species and molecular forms of *Anopheles gambiae* complex (Diptera: Culicidae) collected from Liberia

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The *Anopheles gambiae* complex includes a group of 5 morphologically indistinguishable species which represent the main malaria vector in sub-Saharan Africa. We collected mosquitoes from 3 counties in Liberia every other month from May to November 2011 and from January to May 2012 using 5 different trapping techniques. Molecular techniques were used to identify the *Anopheles* complex using the differences of rDNA region (28S coding region and 5` end of IGS region) between species and the molecular forms of *An. gambiae s.s.* specimens. Over 1,960 *An. gambiae* mosquitoes were collected from all sites and identified. For each specimen, legs were excised; DNA was extracted and tested by multiplex PCR using specific primers for each *An. gambiae* species. RFLP method was performed using HahI restriction enzyme. This enzyme digests specific restriction site located at rDNA region of *An. gambiae s.s.* S form; however, M form remains as a one single fragment due to the absence of this restriction site. Our preliminary results show the wide spread of the *An. gambiae s.s.* in Liberia collection sites. Only 2 *An. melas/An. merus* mosquitoes were detected. We found 72.5% of *An. gambiae s.s.* specimens were S form while 25.4% were M form, 2.8% in the whole collection of specimens were determined to be hybrid M/S.

96 Estimating the shift in population dominance from *Culex restuans* to *Culex pipiens* in northwest Ohio
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Adult *Culex pipiens* and *Cx. restuans* were captured in 14 New Jersey light traps in Lucas County, OH, between May and October, 1980 through 1999. The ratio of *Cx. restuans* to the sum of *Cx. restuans* + *Cx. pipiens* was calculated for each day (pooling traps) for each year. This ratio was regressed over Julian day for each year. Each regression was used to calculate the Julian day on which this ratio was 0.5. This day was the point at which the initial numerical dominance of *Cx. restuans* was overtaken by *Cx. pipiens*, and previously reported as the "*Culex* crossover". The mean crossover date was 201±28, similar to a previously reported value for central Illinois (219). The total cumulative degree-days (above temperature thresholds 0-25°C) were calculated from January 1 to the crossover day for each year. The crossover days were then regressed over their corresponding cumulative degree-days. We found that R-squared values of these regressions declined from 0.933 at a base temperature of 0°C to 0.195 at 25°C; both higher and lower than previously reported for these temperatures, respectively. We suggest that results of analyzing adult population dynamics are somewhat different from those determined from egg rafts reported in earlier studies.

97 Facultative predation and cannibalism in *Prosimulium* black fly larvae (Diptera: Simuliidae) from high elevation sites in Colorado
Layla Al-Shaer, lta312@lehigh.edu, Allison Pierce, Dave Larson and Robert Hancock

Black fly larvae are known to employ filter feeding as their primary mode of nutrient intake. We propose that larvae are opportunistic predators especially in extreme environments. *Prosimulium* larvae were sampled from alpine and subalpine locations in Colorado and gut content analysis revealed evidence of facultative predation and cannibalism. These findings have led us to inquire if there is a correlation between mouthpart morphology and differences in predatory behavior between species.

98 Winter ecology of *Culex pipiens* in New York City
John Zuzworsky, jzuzwors@health.nyc.gov and Waheed I. Bajwa

Culex pipiens plays an important role in West Nile virus transmission in the City of New York (NYC). Individuals of *Cx. pipiens* overwinter as diapausing adults but are rarely found in sufficient numbers to study their ecology in the natural hibernaculum. In NYC, we have identified a number of abandoned buildings, mausoleums, catch basins and hollow bridge abutments, where large populations of diapausing *Cx. pipiens* overwinter. We surveyed and monitored these populations from 2011 through 2012. Our study sites were uninhabited barracks at Fort Totten in Queens. Our routine observations during the winter months revealed population movements within the building as a reaction to changes in temperature and humidity. Diapausing *Cx. pipiens* females initially inhabit these barracks from September through mid-November. Depending on temperature, the survivors break the diapause between March and May and disperse from these hibernacula to reproduce. We have analyzed our data; the results will be discussed in this presentation.

99 Behavioral response of *Aedes aegypti* and *Culex quinquefasciatus* to DEET during the day and night times
Atchariya Prabaripai, faasatp@ku.ac.th, Krajana Tainchum and Theeraphap Chareonviriyaphap

The behavioral responses of the laboratory-bred and field-collected populations of *Aedes aegypti* and *Culex quinquefasciatus* against 5% of standard repellent N,N-diethyl-3- methylbenzamide (DEET) were evaluated using the excito repellency test system. The field populations of *Ae. aegypti* and *Cx. quinquefasciatus* were collected from Kanchanaburi and Nonthaburi provinces, respectively. The behavioral responses of these mosquito populations were performed from 2 periods: day (08.00-16.00) and night (21.00-04.00) times. The numbers of exiting mosquitoes out of the test chambers were recorded within the 30 min exposure time. Results showed that all test populations rapidly escaped after direct contact with DEET. *Culex quinquefasciatus* demonstrated a greater escape activity during the night times.

100 Innovative rearing technique to establish wild caught anopheline colonies in Thailand
Wannapa Suwonkerd, suwannapa@yahoo.com, Somkid Sumlmatr, Nantawan Hengtrakulvenich, Tiphatai Todphet and Theeraphap Chareonviriyaphap

To establish laboratory colonies of wild caught *Anopheles* mosquitoes entails different difficulties and necessities according to species. This study aims to adapt the wild caught anopheline mosquito to artificial environments with their natural vitality little affected. Seven species, *Anopheles minimus A, An. dirus A, An. sawadwongporni, An. pseudowillmori, An. barbrirostris, An. kochi,* and *An. aconitus* wild caught from malaria endemic area in northwestern Thailand were kept to lay eggs in a 9-cm enamel bowl, and larvae were then transferred to plastic trays. We developed a special water system to increase oxygen by filtering and allowing the water to flow over time by using local materials. The results were significant hatchability, pupation rate and very low mortality rate in batches raised in the new water system ($p<0.05$) compared to our current rearing method. This significant advance in mosquito-rearing technique may be adaptable to practically any scale of rearing and to other mosquito species.

Biorational Larvicides and Larviciding II: What Is New in Biorational Larvicides and Larviciding?

101 Microbial mosquito larvicides: The way of application matters!
Min Lee Cheng, mcheng@wvmvcd.org and Tianyun S. Su

Microbial mosquito larvicides have been playing important roles in today's mosquito control. Various formulations mainly customized for different habitats and residual efficacy have been developed and registered using ingredients related to *Bti*, *Bacillus sphaericus* and *Saccharopolyspora spinosa*. The way of application matters significantly in terms of initial and residual efficacy and resistance management. Semi-field and field trials were conducted to evaluate way of application of various microbial larvicides to optimize the outcomes.

102 Breakthrough in feeding zone delivery
Robert Sjogren, rdsjogren@gmail.com

FourStar™ high-density microbial granules provide single brood (SBG) or multi-brood (MBG, CRG) mosquito larval control for up to 60 days via single applications. Flash and sustained release *Bti* and *Bsph*/*Bti* particle combinations are delivered in lethal microbial concentrations from the benthic zone to the larval surface feeding zone. Long-term larval control is achieved by continuous delivery of microbial particles to the feeding zone at optimal concentrations that are ingested throughout larval development and culminate in mortality prior to pupation.

103 Specificity™ – the role and regulation of target-specific solutions for public health
Peter DeChant, Peter.DeChant@valentbiosciences.com, Bala Devisetty, Steve Krause, Jason Clark

Humans have fought insect pests for centuries, but sometimes with tools that didn't reflect the fragile nature of our planet. The introduction of "biorationals" into the US mosquito control industry in the 1970s sparked the notion that target-specific approaches to controlling nuisance and disease vectoring mosquitoes was not only probable, but a sustainable approach that was operationally practical. As the "science of specificity" grew, so did the discovery and development of new biorationals such as *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) by the industry at large. However, it wasn't until relatively recently that scientists, regulatory bodies and health policy institutions began recognizing that target-specific solutions such as *Bti* and *Bs* should be evaluated differently based on major differences between biological organisms and chemicals. Bacterial products such as *Bti*/*Bs* are like all living organisms: no two "cells" are perfectly identical. While genetics plays a crucial role, the manufacturing conditions in which an organism grows can also have a significant impact on its final performance and safety profile. As such, products based on "identical strains" produced, recovered, formulated and controlled under different conditions can have significant impacts on performance, handling and storage stability. Understanding what makes target-specific solutions unique is important to building our knowledge base and development of cost effective and efficacious products for public health vector control in an environmentally sustainable way.

104 Natular® DT - a management tool in organophosphate resistant *Aedes aegypti*
Maria L. Macoris, lulamacoris@hotmail.com, Ima A. Braga, Juliana T. Deus, Vanessa C. Otrera, Adriana F. Araujo, Erika R. Oliveira, Karina Nalon and Maria T. Andrighetti

The biolarvicide Natular® DT was evaluated on semi-field tests to compare residual effect on larvae populations with different levels of susceptibility of the organophosphate temephos. Rockefeller strain, reference of susceptibility; Marília and Salvador: (Resistance Ratio at LC95: 3.5 and 16.7) were exposed in 200 liter water reservoirs. For each population, weekly exposure of 30 third instar larvae on 4 treated (0.50mg/liter, or one tablet per 200 liters) and 2 non-treated (control). Mortality was recorded 24 and 48 h post-exposure. Expositions were performed for 8 wk and 3 complete tests were repeated. A residual effect of 8 wk was the criteria of acceptance for indication of product on the routine of Brazilian National Program for Dengue Control. The results showed an average of mortality superior to 80% for all 3 populations. ANOVA was done to compare results after conversion of mortality to arc-sin values. Paired comparison was done between each 2 populations by Kruskal-Walis. There was no significant difference on populations' response to the biolarvicide, indicating that resistance to the organophosphate temephos did not interfere with the product performance. Results indicate that Natular DT can be considered as an alternative product for management of temephos resistance in *Aedes aegypti* populations.

105 Effectiveness of formulations of Natular® against populations of malaria vector mosquitoes in western Kenya
Edward D. Walker, walker@msu.edu, Nabie Bayoh and John Gimnig

Formulations of the larvicide Natular® were evaluated in controlled field conditions and in 2 villages of western Kenya to determine effectiveness against natural populations of the mosquitoes *Anopheles gambiae*, *Anopheles arabiensis*, and *Anopheles funestus*. Experiments in controlled conditions with natural habitats constructed outdoors involved larvae placed in floating sentinel cages in 5 successive cohorts. Results showed that pupation was completely suppressed for 34 d with a single treatment of the T30 formulation; whereas it was suppressed by 70% at 34 d by the XRG formulation and much less so by the EC formulation which showed 100% effectiveness only to 3 d. A field evaluation was established in 2 villages in Asembo, western Kenya, 1 serving as a control and the other as a treatment. Larval habitats were mapped and sampled biweekly and retreated with T30 and XRG formulations, and adults were sampled in houses. Results showed statistically significant suppression of larval and adult populations in the treated village compared to the untreated village.

106 Novaluron®, a new chitin-synthesis inhibitor
Nayer Zahiri, nayer.zahiri@deh.sccgov.org and Tianyun S. Su

Development and evaluation of more effective insect growth regulators (IGRs) against mosquito vectors have become necessary in order to provide alternate tools to vector control programs. Novaluron® is an insecticide of the class diflubenzoylureas and is an insecticide which inhibits chitin synthesis, affecting the moulting stages of insect development. It acts by ingestion and contact and causes abnormal endocuticular deposition and failed molting. Novaluron has a low mammalian toxicity with low risk to the environment and to non-target organisms. It exhibits promising larvicidal activity against *Aedes*, *Anopheles*, and *Culex* species. The data presents the efficacy of Novaluron against wide range of vectors and pests.

107 Development of an autodissemination approach against cryptic vectors in the South Pacific
Eric W. Chambers, ewchambers@valdosta.edu, James Mains and Stephen L. Dobson

Aedes polynesiensis Marks is the primary vector of lymphatic filariasis (LF) throughout much of the South Pacific, and existing control tools are inadequate. Autodissemination stations have shown promise against cryptic ovipositing *Aedes* species. Toward the development of a novel control tool, the response of *Ae. polynesiensis* to 6 different colors (3 solid fabrics, 2 patterned fabrics, and a plastic tarp) was measured using a digital photographic system. Mosquito-landing frequency was highest for the solid targets (black, navy blue, red) followed in turn by the 2-color pattern targets and the polyethylene target. Mosquito-landing duration was greater for experimental targets when compared to control targets. Mosquito landing frequencies did not change over time during the course of the assay. Insecticidal bioassays using an experimental pyriproxyphen powder formulation (MGK X6932-12) demonstrate high toxicity levels against immature *Ae. polynesiensis* and low toxicity against adult mosquitoes, suggesting that females acquiring insecticidal powder may transmit the insecticide to larval oviposition sites. The results encourage additional investigations evaluating the visual responses of *Ae. polynesiensis* mosquitoes to insecticide-treated targets, with a special emphasis on semi-field and field-based experiments.

108 Strategies for using pyriproxyfen against the Asian tiger mosquito, *Aedes albopictus* (Skuse)
Carl W. Doud, carl.doud@med.navy.mil, Anthony M. Hanley, Raymond M. Platt and Rudy Xue

Container mosquitoes are key threats to the military mission. The Asian tiger mosquito, *Aedes albopictus* (Skuse), is a vector of dengue and chikungunya. The insect growth regulator NyGuard® (active ingredient pyriproxyfen) disrupts adult emergence with lethal results. The objective of this study was to determine the effect of truck mounted ULV spray of NyGuard® against *Ae. albopictus* larvae and adult populations. NyGuard® was applied from a county mosquito control vehicle at 0800 in the morning using a Clarke Cougar ULV truck mounted sprayer applied at a flow rate = 18 oz/min. The treated area had specimen cups containing 10 larvae each placed in the area pre-treatment and the untreated area had control cups also containing 10 larvae each placed pre-treatment. Eight weeks prior to the study, 10 BG-Sentinel traps were placed in the control and treatment areas throughout the study to survey the adult *Ae. albopictus* population. The study was conducted over a 3 ½ month period, during which 3 NyGuard® sprays were conducted. Significant adult emergence inhibition was observed following each spray among larvae in the treatment cups. Water samples were taken from each cup and sent to be analyzed for presence of pyriproxyfen. Results from the water samples and trends in the adult *Ae. albopictus* populations will be discussed.

Latin American Student Competition

109 Detection of dengue (DENV2) serotype 2 infections in field populations of *Aedes aegypti* mosquitoes collected in Quintana Roo, Mexico, 2011
Rosa Maria Sanchez, sanchezcasasrossy@yahoo.com, Esteban Diaz, Rocio Ramirez, Ewry Zarate, Maria Alba Lorono, Ildefonso Fernandez and Adriana Gonzalez

The introduction of dengue virus (DENV) serotype 2 into a new area is widely documented with an accompanying increase of dengue hemorrhagic fever cases, including more severe symptoms and death. Sampling of household, field-collected *Aedes aegypti* populations to identify DENV infections is a useful monitoring system to supplement traditional human serological surveillance when large numbers of asymptomatic cases are prevalent during ongoing dengue outbreaks. Semi-nested RT-PCR was used to identify the dengue virus serotype after mosquitoes were collected with a backpack aspirator. Collections were made in 2 neighborhoods with early cases reported to the State Health Department in Benito Juarez city, Quintana Roo, Mexico, in 2011. Mosquito house densities and geographical locations along with PCR-positive *Ae. aegypti*, were plotted using a free-distribution Google software. Only DENV2 was found in female *Ae. aegypti* collected from 88 households in pooled sampling sites. High vector populations produced 85% house infestation rates with an average of 3.48 ±4.66 to6.02 ±7.30 mosquitoes per house. DENV-2 was detected in 6 (14.6%) out of 41 pools of 10-macerated mosquitoes producing an overall infection rate of 1.4%. Density-dependent relationships and clustering patterns were seen for 83.3% dengue infected mosquitoes.

110 Development of a DNA extraction method from a single *Aedes aegypti* larva, in Cartagena de Indias, Colombia
Doris Gómez-Camargo, degomez@hotmail.com, Yina García-Toscano, Gustavo Mora-García and Yéssika De León

Aedes aegypti is the main vector in the dengue virus transmission cycle. Vector surveillance and control remains the most appropriate strategy for dengue prevention in areas where the disease is endemic. *Aedes aegypti* population dynamics is often influenced by genetic features. However, in Cartagena de Indias, on the Colombian Caribbean Coast, *Ae. aegypti* genomics remains unknown. The aim of this study was to develop a DNA extraction method from a

single *Ae. aegypti* larva, adequate for large-scale studies in low and middle-income regions. A technique was developed according to principles of DNA extraction: 1) mechanical membranes were ruptured, 2) proteins were precipitated (K-Proteinase, NaCl 5M and SDS 10%), 3) DNA was precipitated (ethanol), and 4) rehydration occurred (Buffer TE). The technique developed here was compared to conventional CTAB method. DNA was successfully isolated from a single larva. The final concentration of the solution was 547 µg/ml. The DNA was amplified by end-point PCR. Results were similar to those obtained employing CTAB method. Since this technique required a single larva as biological sample, DNA was rapidly isolated; also low volumes of reagents were used in this technique. Results suggested that this method could be applied in genetic studies of *Ae. aegypti*.

111 Insecticide susceptibility status and resistance mechanisms of *Aedes aegypti* from the Caribbean region of Colombia

Ronald Y. Maestre Serrano, rmaestre22@yahoo.com, Doris Gómez-Camargo, Suljey Cochero, Elkin Monterrosa, Hugo Soto, Zulibeth Florez Rivadeneira, Marcelo Torres, Sergio Goenaga-Olaya, Brenda G. Silva and Adriana E. Flores

We determined the current state of susceptibility to insecticides and biochemical and molecular mechanisms involved in insecticide resistance of 7 sub-populations of *Aedes aegypti* in the Caribbean region of Colombia. Bioassays were performed for temephos in larvae using the WHO method and CDC bottle bioassays for adults with the insecticides lambdacyhalothrin, cyfluthrin, permethrin, deltamethrin, malathion, fenitrothion and pirimiphos-methyl. The resistance ratios were calculated using the susceptible Rockefeller strain as a control. Additionally, the organochloride DDT was evaluated through the WHO impregnated paper technique. Biochemical resistance mechanisms were identified and associated with high level of α, β-esterases, mixed-function oxidases, insensitive acetylcholinesterase and glutathione S-transferases. We identified the mutation Ile1,016 in the gene of the voltage-dependent sodium channel and its frequency. All subpopulations were susceptible to the organophosphates evaluated (RR=1x-5x) except the Puerto Colombia strain which demonstrated resistance to temephos (RR=15x). All subpopulations were resistant to DDT (2-28% mortality). Evaluated strains exhibited resistance values to lambdacyhalothrin between 5-83 fold, for deltamethrin between 1-38 fold, cyfluthrin with 1-34 fold and permethrin from 4-18 fold. Over-expression of glutathione S-transferases were found in all subpopulations with the exception of Puerto Colombia as well as α-esterase in the strains at Valledupar and Monteria and insensitive acetylcholinesterase in the strain from Puerto Colombia. The mutation Ile1,016 was registered in all subpopulations with variability in its frequency.

112 Role of *Kerteszia* species in malaria transmission in two malaria endemic foci in Colombia

Jesús E. Escovar, jeescobarc@unal.edu.co, Martha L. Quiñones, Freddy Ruiz, Richard Wilkerson, Bruce Harrison and Ranulfo González

Species of *Anopheles* in the subgenus *Kerteszia* are characterized by their outdoor biting activity and for developing in bromeliads. The aim of this study was to establish, in 2 malaria endemic zones in Colombia (Tolima and Nariño), which species were present and how these species were involved in malaria transmission, their biting behavior, development sites, relative abundance, natural infection and association with the activities of local residents. In Tolima, 2,487 female mosquitoes were captured, 37 series were reared and 25 male genitalia were analyzed. Morphological and molecular analyses showed that *An. pholidotus* is present as a single species. The other species found in the area was *An. boliviensis*, which forms 2 species (lineages A and B), so far indistinguishable by morphological diagnostic characters. Both species showed predominantly exophagic activity. In Nariño, 4,745 mosquitoes were collected. Samples collected over 24 h periods showed that *An. neivai* was most abundant in the mangroves and vessels (90.8%), while *An. albimanus* represented 82% of the mosquitoes collected indoors. The peaks of activity of *An. neivai* coincided with fishing activities in the marshlands and mangroves. It is recommended that protective measures be implemented to reduce the risk of malaria among fishermen during their activities.

113 *Anopheles* spp. on La Pacurita, Quibdó, Chocó, Colombia

Juan S. Durán, sebastianklon89@gmail.com, Jan Conn and Helena L. Brochero

Pacurita is a small locality 7 km from Quibdó, the capital of Chocó Department (state) in Colombia. It has a hilly topography surrounded by an undisturbed forest environment, and exhibits a monomodal rainfall regime with a short and not well-differentiated dry season. Its population is dedicated to self-sustaining small-scale farming, logging and placer gold mining; the excavations derived from mining seem relevant to the generation of larval mosquito habitats. This 7-month study was aimed at describing the richness and abundance of *Anopheles* spp. in this locality. The oviposition sites were identified and characterized, and the immature forms were collected with standard dipping methods. Female collections were made using the human landing catch method (137 hours indoors and 161 hours outdoors). Adults of 7 species were collected by human landing collections: *An. apicimacula* (n=1), *An. darlingi* (n=21), *An. neivai* (n=6), *An. nuneztovari* (n=187), *An. oswaldoi s.l.* (n=5), *An. rangeli* (n=9), and *An. triannulatus* (n=14). Using ELISA detection of the CS protein, *An. nuneztovari* was preliminarly found infected with *Plasmodium vivax*. This species exhibited 2 peaks of biting activity, one from 22:00-23:-- hours and another from 24:00-01:00 hours, both indoors and outdoors. Only 2 species were found in the oviposition sites, *An. nuneztovari* (n=16, found in 3 excavations derived from gold mining) and *An. darlingi* (n=1). It is strongly suggested the use of long lasting insecticide-treated nets to protect residents from malaria infections.

114 A clinical case of Torsalo myiasis caused by *Dermatobia hominis* (Diptera: Oestridae: Cuterebrinae) from Chiapas, Southern Mexico

Adriana Gonzalez, dramika.ady@gmail.com, Eduardo A. Rebollar-Téllez, Samantha Del Rio, Rosa Maria Sanchez and Ildefonso Fernandez

A 39 year-old male originally from Monterrey, Nuevo Leon, Mexico, traveled frequently to tropical zones and his last trip was in February 2012. The present illness began the last day of the trip to the station Chajul in Chiapas state. He remembered that he was wearing short pants and a short-sleeved shirt. Three weeks later, he observed two furuncle-like injuries on his left leg, one on his right leg and one on his back. The symptoms were pruritus and some slight paresthesia in the wounds. On March 29, the patient observed that something came out from one of the left leg injuries. With intense pressure, he could extract one piece of a fly larva. The patient went to a medical entomology laboratory for a medical examination. He said that he suffered pain, paresthesia and the sense of something moving. In every injury, a central zone about 1 mm in diameter was observed. In the furuncle on his back, a respiratory stigma of a larva was observed. Cooking dough using wheat flour was prepared and smeared on the furuncles and covered with gauze to asphyxiate the larvae. Twenty-four hours later, it was possible to remove a complete larva from every furuncle.

115 Determination of degrees of resistance (DR) to temephos in populations of *Aedes aegypti* in Colombia and their implication in the effectiveness

Gabriela Rey Vega, reyveo@hotmail.com, Martha L. Quinones, Liliana Santacoloma, Ronald Y. Maestre Serrano, Zulibeth Florez Rivadeneira, Luz Adriana Olaya Másmela, Claudia Cabrera, Alberto Díaz and Claribell Hernández

The organophosphates temephos and malathion are the most commonly used insecticides by the Colombian dengue control program. Pressure with temephos has generated resistance in *Aedes aegypti* in some populations but resistance to malathion has not been found, despite its extensive use. The purpose of this study was to determine the susceptibility to these insecticides in 9 dengue endemic areas, the degrees of resistance (DR) and to assess the implication of this resistance in the effectiveness of temephos use in the national dengue control program. Bioassays with adults were done using the CDC bottle bioassay and for larvae, we used the World Health Organization methodology. The implications of the DR in the temephos efficacy in resistant populations were assessed in the laboratory, with and without daily change of water. Biochemical tests of MFO, PNPA, β esterase and AchE modified were performed to detect the mechanism associated with the resistance in this population. The high levels of β - esterase and AchE modified, could be involved in the manifestation of the resistance to temephos in Cundinamarca.

116 Malaria vectors in San José del Guaviare, Amazonia, Colombia

Irene D. Jiménez, pilarjimenez_7@hotmail.com, Jan Conn and Helena Brochero

San José del Guaviare (2°34'15"N -72°38'25"W) is in the Amazonia of eastern Colombia. Malaria is endemic there and drastic changes in the ecosystem such as cutting and burning of the forests for growing crops and the displacement of human residents and indigenous groups from rural to periurban areas in search of better opportunities as a result of the internal armed conflict have led to risk factors that favor malaria transmission in the local population. This study was performed in the periurban area in order to develop prevention strategies and monitor and control of malaria. Mosquitoes were collected using human landing catches indoors and outside dwellings between 18- 6 H for 50 min/h for 3 consecutive nights per month for 8 months. The breeding sites were inspected and the number of malaria cases reported per month was analyzed. Mosquitoes belonging to the *Albitarsis* Complex were identified using the *white* gene and for the first time *Anopheles albitarsis* F was recorded in Guaviare Department (state). Natural *Plasmodium* infections were evaluated in all species collected. The species collected (and percent of collection) were: *An. darlingi* (83.25%) followed by *An. albitarsis* s.l. (8.52 %), *An. braziliensis* (3.86%), *An. oswaldoi* s.l. (0.99%), and *An. rangeli* (0.89 %). *Anopheles darlingi* was caught throughout the night with 2 peaks of biting activity: indoors (20-23H and 01-02H) and outdoors (20-23H and 03-04H). *Anopheles albitarsis* s.l. showed a crepuscular biting behavior with one peak of biting activity indoors and outdoors (18-20H). The fish ponds were the main oviposition sites for *An. albitarsis* s.l. and *An. darlingi*; there was no correlation between adult and larval abundance. Malaria control strategies should be focused on adequate diagnostics, immediate treatment for symptomatic people and control of the immature forms in periurban breeding sites. Adult mosquito control efforts should be undertaken during epidemic situations to interrupt malaria transmission.

Sustainability within the Mosquito Control Industry

117 The greening of society: Why it's not optional

Andrew Winston, andrew@eco-strategies.com

Mr. Winston will discuss why private and public sector organizations are making sustainability a top priority for their operations and strategies. He will explore how global pressures such as resource constraints, technology-driven transparency, climate change, and tough questions from all stakeholders are making sustainability the focus of 21st century business.

118 Sustainability: MMCD's efforts to reduce waste and save energy

Jim Stark, jimstark@mmcd.org

Working smarter and doing more with less is crucial to an organization during tough economic times. The Metropolitan Mosquito Control District (MMCD) has worked at increasing environmental awareness, and staff

continue to identify ways to reduce waste and save energy. Information will be presented on what measures MMCD has taken and how these efforts have affected its social, environmental and financial bottom lines.

119 Harnessing the power of the sun to combat mosquitoes in northeastern Illinois
David Zazra, dzazra@nsmad.com

In 2012, the North Shore Mosquito Abatement District (NSMAD) took a very large step in our sustainability efforts by installing a photovoltaic system designed to provide us with 100% of our electrical energy usage on an annual basis. Information will be presented on the effects this installation has had on our organization and how it complements our efforts to minimize our impact on the environment as we conduct our abatement program.

120 Sustainability: Doing good by doing green
Mark Newberg, mnewburg@central.com and Sue Markgraf

Small changes can make a big difference in the way your operation is perceived by the community. What does sustainability mean to your daily work, how can you incorporate it and how can you present these changes to the community? This session will address practical ways of by doing good by doing green that impacts your operations, offers a proactive opportunity to heighten the awareness of your work and provides value to the public.

Adult Control I

121 Calculating spray efficacy during a public health emergency
Wayne Andrews, wayne@andrews.net and Priscilla Matton

With the increased usage of aerial applications to control public health threats in the United States from both West Nile virus and eastern equine encephalitis emphasis has been placed on the efficacy of these interventions by the public. Various methodologies need to be employed such as enhanced trapping, location of treatment and non-treatment trap sites, collection protocols, calculations and temporal adjustments to trap data. These methodologies need to be followed before, during and after spray applications because they are important for obtaining reliable data. How this data will be used to justify the necessity of the application's expense and risk reduction becomes one of the main goals.

122 Aerial applications of Anvil® 10 + 10 ULV for the control of eastern equine encephalitis in southeastern Massachusetts 2012
Priscilla Matton, brismosqpc@comcast.net

An aerial application of Anvil® 10 + 10 ULV was made to approximately 389,000 acres in southeastern Massachusetts to control eastern equine encephalitis. The application was conducted over 3 nights from July 20-22, 2012. Pre- and post-trapping resulted in a 58% overall reduction in the mosquito population and an 80% reduction in mammal biting mosquitoes. A second application was made on August 13, 2012 to approximately 109,000 acres. Pre- and post-trapping resulted in a 63% overall reduction in the mosquito population and a 75% reduction in mammal biting mosquitoes.

123 Lessons learned in mosquito surveillance and control in Sacramento and Yolo counties, CA, 2005-2012
Paula A. Macedo, pmacedo@sac-yolomvcd.com, Gary Goodman and David Brown

Sacramento County was the epicenter of the California 2005 West Nile virus (WNV) epidemic, and aerial adult mosquito control was necessary over a large urban area. Since then, many changes have been made to our surveillance program, which directs response from our control operations. Our program incorporates targeted surveillance, random surveillance, and increased sampling efforts at hot spots. Despite intensive larviciding and public education to suppress mosquito populations and limit human exposure, aerial adulticiding was necessary in an attempt to interrupt WNV transmission every year since 2005, except for 2009. This presentation will discuss the lessons learned from 2005 to 2012 and challenges faced by the Sacramento-Yolo Mosquito and Vector Control District, California.

124 Impact of nocturnal adulticide applications on *Aedes albopictus* and *Aedes aegypti* populations in New Orleans, LA
Whitney Howe, whowe1@tulane.edu, Robin M. Moudy, Samuel B. Jameson, Krystal Seger, Andrew Ruiz, Claudia Riegel, Dawn Wesson and Sarah Michaels

Aggressive pests and efficient artificial container mosquitoes, *Aedes albopictus* and *Ae. aegypti* are vectors for several medically important arboviruses, including dengue and chikungunya viruses. In New Orleans, LA, surveillance activities have provided insight into the relative distributions of these species in local areas. In the current study, aerial and ground adulticide applications conducted by the New Orleans Mosquito, Termite and Rodent Control Board (NOMTCB) were monitored for their impact on *Ae. albopictus* and *Ae. aegypti* populations. Four grids were established in New Orleans and set with 1 ovitrap per block, filled with deionized water and lined with seed germination paper for oviposition. Water and paper were replaced weekly, and collected eggs and larvae were reared in the laboratory for species identification. Baited BG Sentinel traps were set weekly to sample host-seeking females within each grid. As the majority of mosquito control activities conducted by NOMTCB during the 2012 season were focused on combating the West Nile virus vector *Culex quinquefasciatus*, this study allows examination of the impact of nocturnal adulticide applications on the populations of the diurnally active mosquitoes *Ae. albopictus*

and *Ae. aegypti* and to compare these with adulticide application at dawn. These data can be used to inform future control activities in New Orleans and similar locales, especially in the event of the introduction of an emerging arbovirus into the region.

125 Effectiveness of aerial adulticide applications on area-wide domestic mosquito populations

Christopher Lesser, Christopher.Lesser@manateemosquito.com and Mark Latham

In recent years, *Aedes albopictus* and *Ae. aegypti* populations have become increasingly problematic because of their expanding ranges, increasing population size and threat of disease transmission. These factors have resulted in a sharp increase in the demand for public-agency mosquito control services within residential areas historically not requiring such services. Unfortunately traditional mosquito control techniques have proven to be largely ineffective when applied to domestic mosquitoes for a number of human- and mosquito-behavioral reasons. Towards a continuing effort to improve domestic mosquito control strategies, the Manatee County Mosquito Control District (FL) evaluated the efficacy of 3 aerially-applications of Fyfanon (AI 97% malathion, Cheminova) within a residential community. Effects of these 3 consecutive applications upon the area-wide mosquito population were measured via population dynamics collected in larval ovitraps over 6-month period within treatment and control areas. Results of aerial treatments will be discussed with emphasis upon chemical efficacy, spray-system configuration, and chemical selection.

126 Aerial ULV application of permethrin against adult mosquitoes in an extreme hot-arid zone

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

Aerial ULV insecticide application is an established strategy for adult mosquito control in tropical, hot-humid, or temperate environments. However, not enough is known regarding the efficacy of aerial applications in hot-arid environments similar to those encountered by US military personnel, where mosquito and sand fly disease vectors pose significant threats to force health. We placed colony-reared mosquitoes in sentinel cages across a grid of poles positioned across open and vegetated habitat in the Naval Air Facility El Centro parachute range located in a hot-arid desert borderland area of southern California. Poles were set in separate grids of placement in open sandy areas or directly in low desert vegetation, with sentinel cages attached to poles at the center of creosote bush shrubs. Synergized permethrin was applied in 3 trials using an aerial spray platform aboard a USAF C-130 at ~100 feet altitude at the label rate, and we recorded mosquito mortality in treatment and untreated control areas. In all 3 trials across open sandy areas and in 2 of the 3 trials across vegetated areas, mortality was generally 80-100% at 10 min post-spray. In 1 trial over a vegetated area, mortality was 0 in the majority of cages and ~50% in only 2 cages. Results indicate that aerial application can be effective in a hot-arid environment against mosquitoes, but that even low sparse vegetation may provide refuge from the effects of the insecticide.

127 Evaluation of ground ULV applications against wild populations of *Phlebotomus* sand flies in areas of increased *Leishmania* transmission

Alexandra Chaskopoulou, andahask@ufl.edu, Javid Kashefi, Samiye Demir and Philip G. Koehler

In Greece, visceral and cutaneous leishmaniasis are endemic. The main vector species are *Phlebotomus tobbi*, *P. neglectus*, *P. perfiliewi*, *P. simili*, and *P. papatasi*. No vector control programs are currently in place. In 2011, field studies were conducted in Greece, in areas of increased *Leishmania* transmission, to determine the local sand fly fauna and investigate the behavior of the most important species. Four areas with major sand fly activity were identified (up to 16,000 sand flies/trap/night), and the seasonal activity and flight activity patterns of the sand flies were investigated. In 2012 ground-based ULV trials with a deltamethrin based product were initiated in the areas studied during 2011 (2 treatments sites, 2 control sites). The impact of the treatments on wild sand fly populations were investigated by comparing a) 24 h pre- and post-treatment populations, b) populations between treatments and untreated controls, and c) population dynamics of sand flies in treated and untreated areas during 2011 (no treatment year) and 2012 (treatment year). Treatments resulted in a reduction of up to 98% in wild sand fly populations providing evidence that ULV when applied properly can be an important tool for sand fly management.

128 The environmental variables that influence efficacy of ground ULV applications for adult mosquito management

Jennifer Bodin, jbodin2@gmail.com, Robert Peterson and Mark Breidenbaugh

One of the more effective and widely utilized methods of managing insect vectors of human pathogens is the use of ultra-low-volume (ULV) insecticides. However, comprehensive and systematic studies of how environmental and physicochemical factors affect the efficacy of ground ULV applications of insecticides for adult vector management are lacking. To characterize the factors that influence efficacy, we used permethrin as the active ingredient in numerous field experiments in Louisiana, North Dakota, and Montana because it is the most commonly used material for adult mosquito control and has the broadest range of both water- and oil-based formulations. In this presentation, we will report the environmental and physicochemical factors that affect insecticide efficacy. We will focus on the interactions between formulation attributes, environmental factors, and efficacy.

129 Spatial distribution of droplets on a 25 mm slide

Jacob W. Hartle, jhartle@clarke.com, Grifith S. Lizarraga and Derek Drews

The objective of this test was to create dialogue and questions about the current methods used to manually read 25 mm Teflon coated slides and the anomalies that frequent the collection of droplets. Observations provoked questions as to whether or not droplets have a tendency to collect in certain areas of a slide, and therefore might cause variance to the calculated volume median diameter (VMD) and density on a 25 mm Teflon coated slide.

Insect Repellent Research and Development I

130 Insect repellent mixtures and combinations

Mustapha Debboun, mustapha.debboun@us.army.mil and Kamal Chauhan

The use of arthropod repellent products to prevent mosquito and other arthropod bites is proportional to the public perception of the threat from biting mosquitoes, whether the threat is from annoyance or from their disease risk. Arthropod repellents are usually the first line of defense and give every individual person control over exposure to biting mosquitoes. This presentation will give an overview of the use of arthropod repellent mixtures and combinations and discuss their role in improving efficacy of the repellent products by achieving a broader spectrum of effectiveness.

131 Mosquito bite protection of US Army and US Marine Corps fire-resistant uniforms

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

The US Department of Defense system for personal protection of deployed personnel consists of wearing repellent on the skin and a permethrin-treated uniform to cover the rest of the body. Since 1991, treatment of combat uniforms with permethrin has been done primarily in the field using a 2 gal sprayer, a small spray can, or an individual dynamic absorption (IDA) kit. Since 2009, the US Marine Corps and US Army) have transitioned from 50/50 nylon/cotton uniforms to uniforms 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. Unlike the 50:50 nylon:cotton uniform fabrics, the flame retardant uniform fabrics are constructed more open for breathability to improve wearer comfort. As a result of the increased interstitial space between fibers, there is a greater probability that mosquitoes and other biting flies will bite through the fabric. Incorporation of permethrin 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.

132 Leading mosquitoes up the garden path: a push and pull approach

Ulla Obermayr, ulla.obermayr@biogents.com, Joachim Ruther, Ulrich Bernier, Andreas Rose and Martin Geier

Push and pull strategies take advantage of the fact that insect pests use a variety of semiochemicals to locate mating partners, oviposition sites, and hosts for bloodmeals. Through the use of both deterring and attracting stimuli, the abundance of insect pests can be changed by interfering with the ability of the target pest to find their resource ("push") and luring them to an alternative source where they are trapped and killed ("pull"). Our push and pull approach involves the BG-Sentinel™ trap (BGS) as pull component for *Aedes aegypti* in combination with spatial repellents, e.g., substances which function as attraction inhibitors or interfere with CO_2 perception. We used Y-tube olfactometer assays to measure behavioural responses of host seeking *Ae. aegypti* females towards a finger or CO_2 both in the presence and absence of different test compounds. A room test set-up with a simple push and pull situation helped to investigate the spatial potential of the most promising compounds under realistic conditions, with free-flying mosquitoes and a human bait. Best results were obtained when a 10% mix of catnip (*Nepeta cataria*) and homopiperazine was tested in combination with the BGS trap: compared to control trials human landing rates could be reduced by 44%.

133 Trifluoromethylphenyl amides as novel insecticides and fungicides

Maia Tsikolia, maia.tsikolia@ARS.USDA.GOV , Ulrich R. Bernier, Natasha M. Agramonte, Nurhayat Tabanca, David E. Wedge, Katelyn C. Chalaire, James J. Becnel, Gary G. Clark, Kenneth J. Linthicum, Monique R. Coy, Daniel R. Swale and Jeffrey R. Bloomquist

Because of increased resistance to insecticides in arthropods, it is necessary to identify new chemicals that may have novel modes of action. Following an extensive literature search for compounds with insecticidal and mosquito repellent activity, we have designed and synthesized a set of 20 trifluoromethylphenyl amides. These compounds were evaluated for toxicity against *Aedes aegypti* larvae and adults, for repellency against female *Ae. aegypti* and *Anopheles albimanus*, and for fungicidal activity against fungal species. Selected compounds were evaluated for toxicity against *Drosophila melanogaster* and, unlike fipronil, showed full activity against a resistant strain having an altered gamma-aminobutyric acid (GABA) receptor. The most potent compounds from this initial evaluation were used to design and synthesize 14 additional derivatives. From the bioassays of these amides, 2 compounds, *N*-(3-(trifluoromethyl)phenyl)acetamide and *N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)-2,2,3,3,3-pentafluoropropanamide, repelled *Ae. aegypti* at lower concentration than DEET. These same 2 compounds and 2-chloro-*N*-(2,6-dichloro-4-(trifluoromethyl)phenyl)acetamide exhibited comparable fungicidal activity to the

standards, azoxystrobin and captan, against *Phomopsis viticola* and *Colletotrichum acutatum* fungi. In the future, we plan to conduct a QSPR study using the insecticidal activity of all 34 trifluoromethylphenyl amides as the properties for input into the model.

Latin American Symposium III

134 Field efficacy of repellent formulation containing para-menthane-3,8-diol and lemongrass against *Culicoides pachymerus* (Diptera: Ceratopogonidae) in Colombia

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Culicoides pachymerus causes a big nuisance to the inhabitants of the western region in Boyacá department (state) of Colombia. This study evaluated the effect of a repellent lotion based on p-menthane-3,8-diol (16%) and lemongrass oil (2%) against the bites of *C. pachymerus*. The repellent lotion was compared simultaneously with a control (no treatment) by human landing catches of *C. pachymerus* on forearms of volunteer pairs located around houses. Protection percentage and protection time for 3 to 6 h after repellent application was calculated. The test was repeated 10 times. Only 2 female *C. pachymerus* were caught on the repellent treatment. In contrast, the mean biting rate in the control was 47.7 midges/person/10 min. The mean protection percentage of the repellent was 100% up to 4 h and 99.5% up to 5 h. Protection time was 332.2 and 338.2 min in the 2 replicates where bites of *C. pachymerus* were confirmed. In the remaining 8 replicates, protection time overcame the test duration. The evaluated repellent showed high efficacy against *C. pachymerus*, up to 5 h post-application.

135 Insecticide activity of four essential oils on adult female *Anopheles albimanus* (Diptera: Culicidae) in experimental conditions

Estrella Cardenas, cardenases@hotmail.com, Isabel Riveros and Ligia Lugo

Anopheles albimanus is widely distributed in the Neotropics and is the main vector of malaria. The essential oils of *Cymbopogon citratus* (lemongrass), *C. nardus* (citronella grass), *E. globulus* (eucalyptus), and *E. caryophyllata* (clove) were tested for their insecticidal activity against female *An. albimanus*. The bioassays were realized on female *An. albimanus* of a reference colony at the Instituto Nacional de Salud of Bogotá where the colony had been maintained in the Entomology Laboratory since 1976. The oils to be tested were diluted with mineral oil USP to concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% v/v. The bioassays were performed following the WHO methodology. The exposure time was 60 min for 20 females with 5 repetitions per concentration. The laboratory temperature was 28° ± 2°C. Concentrations between 2% and 2.5% of *C. citratus*, *C. nardus* and *E. globulus* oils showed high insecticidal activity against *An. albimanus*, compared to *E. caryophillata* oil. Values for Chi-square demonstrated that the mortalities produced by each essential oil were associated with the concentration used. The LC_{50} of *C. citratus*, *C. nardus* and *E. globulus* oils was 0.7%, 1.0% and 1.9%, respectively while the LC_{90} was 1.3%, 1.83% and 3.3%, respectively. The LC_{50} of *E. caryopyllata* oil was 4.7% and the LC_{90} was 6.7%. The *C. citratuas*, *C. nardus* and *E. globulus* oils showed high insecticidal activity against *An. albimanus*; while the *E. caryophillata* oil at the concentrations tested showed no insecticidal effect for this mosquito species.

136 Toxic effect of four essential oils on female *Aedes aegypti* (Diptera: Culicidae) in experimental conditions

Estrella Cardenas, cardenases@hotmail.com

Aedes aegypti is the vector of the four viruses that cause dengue fever; one of the major diseases in tropical areas. The objective of this study was to evaluate the toxicity of essential oils of the following plants: *Cymbopogon citratus*, *C.nardus*, *Eucaliptus globulus* and *Eugenia caryophyllata* on female *Ae. aegypti* (Rockefeller strain). The oils to be tested were diluted with mineral oil USP to concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% v/v. The bioassays were performed following the WHO methodology. The exposure time was 60 min for 20 females and five repetitions were performed per concentration. The laboratory temperature was 28 ± 2 degrees C. Concentrations between 2% and 2.5% of *C. citratus* and *C. nardus*oils showed high insecticidal activity against *Ae. aegypti*, compared to *E. globulus* and *E. caryophillata* oils. Values for Chi-square demonstrated that the mortalities produced by each essential oil are associated with the concentration used. The LC_{50} of *C. citratu*s and *C. nardus* oils was 1.07% and the LC90 was 2.01% and 1.96%, respectively. The LC_{50} of *E. globulus* and *E. caryopyllata* oils was 8.53% and 5.23, respectively, and the LC_{90} was 14.96% and 8.03%, respectively. The *C. citratuas* and *C. nardus* oils showed high insecticidal activity against *Ae. aegypti*; while the *E. globulus* and *E. caryophillata* oils with the concentrations tested no showed insecticide effect on *Ae. aegypti*.

137 Efficacy of methoprene (Altosid 1.3G) against *Aedes* spp. in Emiliano Zapata, Cihuatlan Municipality, Jalisco, Mexico

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Dengue fever is a serious public health problem and increasingly difficult to control. This is due in part to adaptation of mosquitoes to climates at higher elevations and tolerance to the insecticides commonly used for mosquito control. In the state of Jalisco, Mexico, there is a high incidence of dengue: 4,919 confirmed cases were reported in 2009. This field study was initiated to determine the possibility of controlling mosquitoes that vector dengue fever (*Aedes aegypti*) using Altosid 1.3G. The study was conducted through 2 full mosquito cycles in the town of Emiliano Zapata, Cihuatlan Municipality in Jalisco. Ovitraps were used as the monitoring tool of choice. Twenty-one ovitraps were distributed throughout the town of 919 homes. Cisterns and other water-holding receptacles used for drinking water

were identified as a major source of mosquito oviposition and were treated with Altosid. Quantitative data was gathered using the methodology described in the CENAPRECE guide for ovitraps. Overall efficacy was 86.13% based on the number of eggs laid pre- vs. post-treatment. Dosing of the product was straightforward, and did not instill any color, taste or odor to the treated water as is common with organophosphates. The community welcomed the Altosid 1.3G treatment for these qualities. During the treatment period, the larvicidal control was so successful that routine adulticiding was not required.

138 Culicidae morphometric studies I: Is *Psorophora pruinosa martini* a valid species?
Aldo Ortega, agrortega@hotmail.com, Adelfo Sánchez, Félix Ordóñez, Ma. Teresa Valdés and Francisco Sánchez

In 1935, E. Martini published "The Mosquitoes of Mexico," which is a classic, useful and necessary literary source for any study of mosquito distribution in Mexico. *Psorophora (Grabhamia) pruinosa* is described by the author and some distinctive characters which allow differentiation from a very similar species, *Ps. (Gra.) signipennis* (Coquillett) are provided. In 1965, A. Díaz Nájera discussed the validated specific level of *Ps. pruinosa* and proposed that *Ps. pruinosa* be changed to synonymy level of *Ps. signipennis*. However, *Ps. pruinosa* is still considered a different taxon from *Ps. signipennis* in many mosquito catalogues of the world. We collected both, immature and adult stages of *Ps. pruinosa* in Torreón, Coahuila, México, and did not observe morphometric differences to separate the taxon from *Ps. signipennis*.

139 Culicidae morphometric studies II: The identity of *Toxorhynchites* in northeastern Mexico
Aldo Ortega, agrortega@hotmail.com, Adelfo Sánchez, Félix Ordóñez, Ma. Teresa Valdés, Francisco Sánchez and Thomas Zavortink

Through time, *Toxorhynchites* taxonomy in northeastern Mexico has been doubtful. Several historical publications that make reference to the presence of this genus in the states of northeastern Mexico (i.e., Coahuila, Nuevo Leon, and Tamaulipas) record the occurrence of taxa such as *Tx. moctezuma* Dyar and Knab, *Tx. rutilus septentrionalis* (Dyar and Knab), and *Tx. theobaldi* (Dyar and Knab). Since the elevation from synonymy with *Tx. theobaldi* (Zavortink and Chaverri, 2009), the identity of species that occur in northeastern Mexico becomes clearer. More than 100 adult *Toxorhynchites* specimens deposited in the Culicid Collection of the Universidad Autonoma Agraria "Antonio Narro" Unidad Laguna and collected in northeastern Mexico were reviewed. These were consistent with *Tx. moctezuma* description. *Toxorhynchites rutilus septentrionalis* is not present in northeastern Mexico.

140 Detection of mutation (F1534C) in the voltage-gated sodium channel in *Aedes albopictus* from northeastern México
Gustavo Ponce, gponcealfa@gmail.com, Iram Rodriguez, Selenne Garcia-Luna, Derek Jimenez and Adriana E. Flores

The Asian tiger mosquito, *Aedes albopictus*, is the principal vector of chikungunya virus and secondary vector of dengue virus. We collected *Ae. albopictus* from northeastern México for detection of mutations in the voltage-gated sodium channel. We detected amino acid substitution F1534C, which is suspected to confer knock down resistance (kdr) to pyrethroid insecticides. Total DNA was isolated using the DNeasy tissue kit following manufacturer's protocols (Qiagen), the purity and integrity of DNA was assessed using standard spectrophotometry methods (NanoDrop, Thermo Fisher Scientific, Wilmington, DE) and agarose gel electrophoresis, respectively. The PCR products were analyzed in a 1% agarose gel stained with ethidium bromide and visualized under UV light. PCR reactions were digested with a mixture of exonuclease I (EXO) and shrimp phosphatase (SAP) (1:2). The sequencing reaction was conducted with Big Dye Terminator (Applied Biosystems) according to manufacturer's instructions. Primers used for amplifying and sequencing were previously reported. The sequences obtained were aligned using ClustalW and compared with reference sequences.

141 KDR mutations *Ile*1,016 and *Cys*1,552 associated with pyrethroid resistance in *Aedes aegypti* from Venezuela
Leslie Alvarez, hleslieag@hotmail.com, Gustavo Ponce, Karla Saavedra, Milagros Oviedo and Adriana E. Flores

Mutations *Ile*1,016 and *Cys*1,552 present in the voltage-gated sodium channel gene have been associated with resistance to pyrethroids in *Aedes aegypti*. We collected and analyzed populations from western Venezuela during 2008, 2010 and 2012 and determined the frequency of *Ile*1,016 and *Cys*1,552 using real time-PCR. Also, we analyzed a population (Pampanito) selected for 15 generations with deltamethrin at a dose of 0.17 µg. Mutation *Ile1,016* was present with frequencies from 0.01 to 0.37 and mutation *Cys*1,552 with frequencies of 0.35 to 0.92 in strains analyzed. The Pampanito strain showed a frequency of *Ile*1,016 at F1 of 0.02 and increased to 0.5 at F15 and 0.35 to fixation for *Cys*1,552, after selection with deltamethrin. Our results demonstrated that the frequency of *Cys*1,552 is higher in natural populations of *Ae. aegypti* from western Venezuela compared with the *Ile*1016. We found that deltamethrin selected *Cys*1,552 more rapidly than *Ile*1,016 mutation.

142 Synergist efficacy of S,S,S,-tributylphosporotrithioate (DEF) and piperonyl butoxide (PBO) with permethrin on resistant strains of *Aedes aegypti* (Diptera: Culicidae)
Adriana E. Flores, adrflores@gmail.com, Wilfredo Arque-Chunda, Juan M. Ballesteros, Jesus E. Castrejon, Jesus A. Davila, Adriana Gonzalez, Laura Mayela Montes, Kenzy I. Peña, Jorge J. Rodriguez, Selene M. Gutierrez and Gustavo Ponce

Pyrethroid resistance can be considered one of the main problems to the control of many mosquito vectors of diseases. The use of synergists is one of the strategies used to help increase the efficacy of certain insecticides. This investigation demonstrates the efficacy of S,S,S,-tributylphosphorotrithioate (DEF) and piperonyl butoxide (PBO)

with permethrin against 2 strains of *Aedes aegypti* with high resistance levels 18-fold of $RRKD_{50}$ (knockdown resistance) and 12-fold of $RRCL_{50}$ (24h post-recovery resistance) to this pyrethroid. Mosquito larvae were used for testing the synergistic effect. DEF was used at a concentration of 0.03 mg/l and for PBO stock solutions of permethrin and PBO were mixed in 1:6 ratio. The synergistic ratio and the percent suppression in permethrin tolerance were calculated by using LC_{50} values. The results show that DEF is more effective synergist against the 2 strains used in this investigation compared with PBO. The results also suggests the role of nonspecific esterases as resistance mechanisms to permethrin.

143 Status of the insecticide susceptibility in five populations of *Aedes aegypti* in Caldas, Colombia
Andrea M. Conde, marcelaconde79@gmail.com, Cesar A. Castellanos and Martha L. Quinones

In a previous study in 2006 and 2007, *Aedes aegypti* showed resistance to temephos, with DR (degrees of resistance) of 13.27 and 11.28 in 2 localities (Las Ferias and Las Margaritas, respectively) in the municipality of La Dorada (Caldas). Given that the dengue control program uses temephos routinely in the whole department (state) of Caldas, an update on the susceptibility levels in other neighboring endemic areas was needed. Bioassays were carried out using the WHO tests of larvae and the CDC bottle methodology in adult *Ae. aegypti*. Tests were conducted with 5 insecticides (temephos, malathion, pirimiphos methyl, fenitrothion and deltamethrin) in mosquito populations from Las Ferias, Las Margaritas, Victoria, Marquetalia and Viterbo. CDC bioassays showed susceptibility to deltamethrin, malathion, fenitrothion in all tested populations. The populations of Las Ferias, Las Margaritas and Victoria reported resistance to pirimiphos methyl with mortalities between 80% and 98%. WHO bioassays showed temephos susceptibility in Viterbo, Las Ferias and Marquetalia. Two populations (Las Margaritas and Victoria) showed DR of 5.6 and 5.5, respectively. A reduction in DR was observed in the populations previously evaluated in 2007, probably due to a reduction in temephos use in the area.

Operations/Management

144 West Nile....Texas style
Fran Krenick, FKrenick@clarke.com, Clark Wood and Caleb T. Stitely

In the worst West Nile virus outbreak since 2003, emergency response was anything but service as usual. This paper discusses the significant challenges and demands decision makers and cooperators dealt with in the 2012 emergency aerial spray.

145 Where source reduction is not an option II: a report from Williston, ND documenting challenges and opportunities in a rapidly changing area
Francis Bosch, franb@co.williams.nd.us

Four consecutive years of extensive aerial larviciding over the confluence areas of the Yellowstone, Missouri, and Muddy rivers has brought about significant reductions in both nuisance and vector species. The Williston Vector Control District continues to explore new control methods such as controlled burning by collaborating with the various government agencies managing the natural resources in the area. This is often accomplished for pennies on the dollar. Industries located here have joined the fight with contributions of equipment and the District still receives monetary donations from many of its residents along with their payments. As oil drilling intensifies in the region, Williston is experiencing rapid growth and demographic changes which usually happen over decades rather than a few years' time. Despite major flooding, growing pressure on infrastructure and environment, and the cancelation of a major spray program, there were unexpected opportunities that balanced the challenges.

146 Integrating technology into integrated vector management (IVM)
Joel Buettner, joelb@placermosquito.org

The use of scientific data to make effective and efficient treatment decisions is the core principle of IMM. Advancements in surveillance, treatment and GIS technology have created the ability to create and record vast amounts of data compared to what was possible in the recent past. The ability for a MVCD to collect and analyze data to make decisions, and report findings to regulators, trustees, and the public has never been more important. For well over a decade, many Districts have incorporated database and field data collection solutions at various levels to improve their IMM programs. Starting in 2010, Placer MVCD has undergone an extensive project to integrate information technology and business intelligence into its program at a fundamental level. This process has sparked the discussion of the continually changing opportunities for technology to enhance operations to protect human health at many levels namely: 1) data collection, 2) analysis, 3) decision making, and 4) reporting and accessibility. At a larger scale, developments in information technology, and the associated opportunities in operational efficacy and efficiency, are foreshadowing the evolution of how informational technology systems may better serve all vector control agencies in the future. While most MVCDs collect data, improvements in how to use and disseminate and make operational decisions based on the data will help to improve IVM into the future.

147 She's back...the rediscovery of *Aedes aegypti* in Volusia County, FL
Edward D. Northey, enorthey@volusia.org and James McNelly

This presentation will focus on the recent discovery and spatial distribution of *Aedes aegypti* within Volusia County. We will focus on events ranging from a mosquito control inspector's discovery during a customer service request,

taxonomical data from recently submitted larval samples and adult aspirations (field collected), the use of GIS as a tool for surveillance and the role of interns as a resource to Volusia County Mosquito Control.

148 Current issues in saltmarsh management in Florida

Douglas B. Carlson, doug.carlson@irmosquito2.org

In Florida, the management of salt marshes and mosquito control impoundments is an important part of many agencies' IMM program. Florida's Subcommittee on Managed Marshes, a legislatively-established committee made up of a diverse group of individuals involved in saltmarsh management, serves as a forum for working to blend mosquito control and natural resource interests in these environmentally sensitive habitats. Current issues discussed in this presentation include recent regulatory challenges along with increased pressure to reduce costs in the management of these ecologically important areas.

149 A reduction in your budget can improve your program's operational effectiveness and efficiency

Mark E. Smith, mmcd_mes@mmcd.org

Current economic challenges are demanding that mosquito control programs do more with less. A critical review of your program provides the opportunity to increase the effectiveness and efficiency of your operations while cutting costs. The Metropolitan Mosquito Control District has focused on using innovation to continuously improve our processes, stretch our budget and provide better service to our customers.

150 Framework for excellence: mission, vision, values

Michael C. Herrmann, cherrmann@mmcd.org, Diann Crane and Jim Stark

Key components of an organization's strategic plan are its mission, vision, and value statements. Stated another way: what do you do, why are you here, and who are you? Our organization developed its mission and vision statements 20 years ago. To remain relevant in today's fast-changing world, we needed to update our mission and clarify our vision statements. We also wanted to develop a value statement—an agreed upon set of values we can use in our daily activities. Staff representing all aspects of our organization assembled to work on this task. A banner depicting these three components was placed in each field office to help employees fully understand our guiding principles. This presentation will outline the steps taken to ensure the Metropolitan Mosquito Control District has clear, understandable, and mutually agreed upon goals that best represent our organization. This framework of clearly defined mission, vision, and organizational values will guide us in future years.

151 The global plan for insecticide resistance management

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Global malaria control efforts have achieved significant gains. Between 2000 and 2010 malaria mortality dropped an estimated 25% globally and 33% in Africa. Much of the success is due to vector control, especially the scale-up of long lasting insecticidal nets and indoor residual spraying. The emergence of insecticide resistance, particularly pyrethroid resistance, threatens to undermine these gains. In May 2012, the World Health Organization and the Roll Back Malaria Partnership launched the "Global Plan for Insecticide Resistance Management", GPIRM, after consultation with a broad range of partners from national programs, academia, the private sector, donors and implementing partners, including the US President's Malaria Initiative, implemented by USAID and CDC. GPIRM lays out a global strategy along 5 pillars, with opportunities for collaboration with AMCA and its membership: a) establish national insecticide resistance management strategies; b) build capacity for national entomological monitoring and data management; c) develop new, innovative vector control tools; d) fill knowledge gaps on resistance mechanisms and impact; and e) ensure enabling mechanisms are in place. Insecticide resistance is a serious threat; it is through collective action, particularly with members of the AMCA, we must build national human capacity and systems to implement monitoring and mitigation strategies, find new tools and paradigms to maintain the gains and achieve our goals.

Adult Control II

152 Effectiveness of bifenthrin and deltamethrin as barrier treatments for controlling *Aedes taeniorhynchus* in Key Largo, FL

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The effectiveness of residual barrier treatments for long-range mosquito species such as *Aedes taeniorhynchus* in harborage and interception zones was evaluated to determine the level of control achieved and the longevity of the treatments. This study investigated whether residual barrier treatments are an appropriate technique for minimizing adult mosquito density in adjacent residential and recreational areas where the option of broad-scale space spraying such as ground ULV and aerial adulticiding have been reduced or removed, thus reducing pesticide application costs and hopefully minimizing impacts to non-target organisms harboring near target mosquito populations. Characterization of A-1 Mist Sprayer was performed to provide optimal residual distribution throughout the target canopy. Two pyrethroid insecticides, bifenthrin (7.9%) and deltamethrin (4.75%), were applied at their maximum label concentrations as barrier treatments and residual efficacy for reducing adult mosquito populations was evaluated and compared to mosquito populations in untreated areas. Adult populations were measured daily using landing rate counts and once weekly using CO_2-baited American Biophysics Corporation light traps. In addition to population monitoring, randomly treated vegetation underwent no-choice bioassays with

field caught *Ae. taeniorhynchus* from untreated areas in order to confirm residual efficacy of both insecticides 15 days post treatment.

153 Sand fly control in Kenya with residual pesticide application on HESCO barriers

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US military operations in hot-arid regions still face significant impacts from mosquito and sand fly vectors of disease. Personal protective measures (PPM) such as DEET or treated bed nets and clothing can reduce contact with disease vectors and nuisance insects; however, irregular use of PPM coupled with widespread substandard sanitation in operational environments result in routine exposure to insect threats. We investigated a technique using standard-issue military pest management spray equipment and pesticide formulation to reduce insect threat loads in a hot-arid region regardless of PPM. HESCO blast wall barriers are ubiquitous in current US military theaters of operation in Afghanistan and the Arabian Peninsula. HESCO units are constructed of a rugged cloth-like material on which we hypothesized a residual pesticide treatment would be effective and long-lasting. We applied an aqueous solution of λ-cyhalothrin at the label rate using a backpack mist blower to small, enclosed structures made of HESCO units in a hot-arid region in western Kenya targeting local abundant populations of *Phlebotomus* sand fly vectors of *Leishmania*. Periodic samples cut from the treated material were shown to cause high mortality for at least 3 months against mosquitoes in laboratory bioassays, and the treatment also significantly reduced wild sand fly densities in traps placed inside the units in the field for at least 5 months post-treatment.

154 The effect of Uvitex OB on pesticide toxicity

Jane A. Bonds, jasbonds@gmail.com, Brad Fritz and Wesley C. Hoffmann

Fluorescent tracers are employed to characterize the downwind dispersal of pesticide used in public health space sprays. Recent field trials showed poor correlation between caged mosquito mortality and pesticide concentration. This raised the question, could the fluorescent tracer degrade the toxicity of the pesticide? Studies were conducted therefore, under controlled conditions to investigate the effects of the fluorescent tracer Uvitex OB on insecticide toxicity. Using a cage bioassay wind tunnel the effects of Uvitex on malathion, permethrin, sumithrin and etofenprox were investigated. Although not significant as a result of within-group variation, the results showed a consistent trend erring toward Uvitex OB increasing not decreasing the toxicity of the pyrethroids and the non ester pyrethroid. The results will be discussed.

155 Effectiveness of malathion 40%, using a dosage of 132 g AI/ha to control oviposition activity of *Aedes aegypti* in Chetumal, Quintana Roo, México

Rafael Alpuche, ralpuche@hotmail.com, Marco Dominguez, Jorge Esparza, Pedro Mis, Ildefonso Fernandez and Alejandro Villegas

The effectiveness of ultra-low-volume (ULV) applications of 40% malathion to control oviposition activity of *Aedes aegypti* was demonstrated in Chetumal, Quintana Roo, from July to September 2012. Cycles of 3 applications during 1 ½ day were applied weekly since epidemiological weeks 26 to 38, using a London Fog heavy duty cold fogger. An area of 3,500 ha was treated, which included the outskirts and the entire city of Chetumal, complying with the current list of insecticides recommended by CENAPRECE against vector-borne diseases for 2012. The average number of eggs per ovitrap of adult *Ae. aegypti* was reduced significantly after starting the ULV applications of malathion. The reduction ranged from 14.8 eggs per ovitrap in week 26, to 1.99 eggs per ovitrap in week 38. Likewise, the tendency of dengue cases in Chetumal shows a declining tendency in the last 14 wk correlated to the use of malathion in this year. Rates of other mosquito species were reduced up to 90% of bites/men/night. Our results indicated that ULV treatments with malathion 40% (Lethal Mist) applied by ULV spraying London Fog can be used to control *Ae. aegypti* during outbreaks of dengue hemorrhagic fever. In addition, this is a water-based formulation, which has shown to be more environmental friendly, less corrosive and safer for human beings.

156 Widespread cross-resistance to pyrethroids in *Aedes aegypti* (L.) from Mexico

Adriana E. Flores, adrflores@gmail.com, Gustavo Ponce, Brenda G. Silva, Selene M. Gutierrez, Cristina Bobadilla, Beatriz Lopez, Roberto Mercado and William C. Black IV

Seven F1 strains of *Aedes aegypti* (L.) were evaluated by bottle bioassay for resistance to 8 pyrethroids. The New Orleans strain was used as a susceptible control. Mortality rates after a 1h exposure and following a 24 h recovery period were determined. The resistance ratio between the 50% knockdown values ($RRKD_{50}$) of the F1 and New Orleans strains indicated high levels of knockdown resistance (kdr). The $RRKD_{50}$ with α-cypermethrin varied from 10-100 among strains indicating high levels of kdr. Most of the strains had moderate resistance to d-phenothrin. For z-cypermethrin and bifenthrin, only 1 strain exhibited resistance with values of 10- and 21-fold, respectively. None of the strains showed $RRKD_{50}$ >10 with deltamethrin, and moderate resistance was seen in 3 strains, while the rest were susceptible. Mosquitoes from all strains exhibited some recovery from all pyrethroids except d-phenothrin. Regression analysis was used to analyze the relationship between $RRLC_{50}$ and $RRKD_{50}$. Both were highly correlated so that the slope could be used to determine how much additional pyrethroid was needed to insure lethality. Both $RRLC_{50}$ and $RRKD_{50}$ values were highly correlated for all pyrethroids except bifenthrin indicating strong cross-resistance.

157 Bottle bioassays, Florida Keys
Lawrence Hribar, lhribar@keysmosquito.org

Aedes taeniorhynchus remains susceptible to permethrin, resmethrin, bifenthrin, and malathion. Deltamethrin appears problematic; studies are ongoing. *Aedes aegypti* appears to be developing resistance to bifenthrin.

158 Insecticide resistance management
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An outreach program focused on managing insecticide resistance is under way in New Jersey. Standardized procedures such as the CDC bottle bioassay and the WHO larval assay are the basis for measuring the response of targeted mosquito species to "off-the-shelf" insecticides. Hands-on workshops and on-line resources are being utilized to disseminate information about techniques and protocols for preparing "diagnostic concentrations" of active ingredients to distinguish between "susceptible" and "resistant" populations. Standardized procedures promoted by international organizations such the Insecticide Resistance Action Committee and World Health Organization Pesticide Evaluation Scheme are the foundation of the program. The long-range goal is to increase the participation of New Jersey mosquito control programs in regular, systematic (at least twice a year) monitoring of insecticide resistance. Decisions on choice of active ingredient, frequency of rotation, application rates must be based on scientifically collected data. Examples of programs that have implemented such procedures will be presented. Case studies of programs that have failed to follow these guidelines will be discussed. A primer on the rationale of the long-range program will also be provided.

159 The use of DDT for vector control: the Zambian experience
Makusa Dayton, daytonm@zambiaissp.org, Chadwick Sikaala and Musapa Mulenga

Indoor residual spraying (IRS) forms part of the main malaria prevention strategy in Zambia and central to this strategy in the use of DDT. IRS activities using DDT have been carried out in Zambia since 2001. DDT has been an insecticide of choice in Zambia because of its ability to remain on the surface of highly porous sprayable surfaces unlike the other insecticides that get absorbed in the wall making the application futile. The relative low cost and long residual effect clearly make it a darling to vector controllers. Its low mammalian toxicity and inability to cause skin reactions as compared to other insecticides makes it more acceptable to householders and thus accepting of the intervention. The use of DDT for IRS has been scaled-up to 15 districts protecting about 3.28 million vulnerable people against malaria. This has been made possible through the support of the USAID funded HSSP and RTI programs. While HSSP with other partners have provided training to districts in spraying technique, judicious use of chemicals, insecticide stock management and environmental and personnel safeguards, and support for improved storage and the disposal of DDT waste, RTI supports the procurement of DDT, construction of evaporation tanks and environmental monitoring of DDT. Malaria incidence has shown a marked decline in the areas sprayed with DDT. Deaths have reduced, and malaria slide (RDT) positivity has also reduced. Challenges include environmental compliance, waste disposal and stringent international requirements for use of and the demonization of DDT.

Insect Repellent Research and Development II

160 Comparative efficacy of the plant-based repellent F4302 to mosquitoes and ticks
Brooke Bissinger, bbissinger@tyratech.com, Jason Schmidt, John J. Owens, Sheila Mitchell and M. K. Kennedy

Repellent efficacy of F4302 (5% geraniol) was compared to 16 other products in laboratory arm-in-cage trials against *Aedes aegypti*. Half of the products exhibited a mean repellency below 90% at 0.5 h after application. F4302 demonstrated 94.6% repellency 5 h post treatment, which was a longer duration than any of the other repellents tested. The positive control, 15% DEET was repellent for 3 h before activity dropped below 90%. F4302 provided 95.2% repellency 6 h after treatment in laboratory trials against *Anopheles quadrimaculatus,* while DEET was 72.2% repellent. In field trials in North Carolina, where the primary mosquito was *Aedes albopictus*, F4302 provided 100% repellency at 5 h compared to 77.6% repellency by DEET. F4302 was compared to DEET (15%) in laboratory two-choice bioassays against *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*, and *Rhipicephalus sanguineus*. At 2.5 and 3.5 h after treatment of filter paper with F4302, 100% repellency was observed for all species with the exception of *I. scapularis* at the 3.5 h evaluation where repellency was 95.8%. A slight variant of F4302, F4228 was compared to DEET in field trials in North Carolina. Significantly fewer ticks (predominantly *A. americanum*) were collected from socks worn by human volunteers treated with F4228 compared to those treated with DEET. Repellencies for F4228 were 89.6 and 70.4% at 2.5 and 3.5 h, respectively while DEET was 51.9 and 18.1% repellent at the same time points.

161 Comparison of two mosquito bioassay methods for the estimate of minimum effective dose in repellents
Natasha M. Agramonte, nme@ufl.edu, Ulrich R. Bernier and Salvador A. Gezan

It is expected that laboratory-based repellent bioassays should reliably evaluate the efficacy of compounds that deter mosquito feeding behavior. The variety of repellent bioassays available allows for flexibility in design but makes it difficult to compare any two methods, including *in vitro* and *in vivo* comparisons. The most reliable data come from skin assays; however, this exposes volunteers to chemicals and mosquito bites. In this study, 4 repellent active ingredients were used: DEET, IR3535, picaridin and para-menthane-3,8-diol. Results from bioassays with a module-based method were operated *in vitro* with a membrane and *in vivo* on the skin of the leg and were then compared to an *in vitro* method where repellent treated cloth is placed over an arm that is inserted into a cage of mosquitoes.

Pooled data from 6 volunteers were used to estimate effective doses for 4 repellents at the 50, 95, and 99% levels (ED_{50}, ED_{95}, ED_{99}) using a dose response curve with a probit model in the module tests. The ED_{99} was estimated with repellent-treated cloth as the concentration that prevented 99% of the mosquitoes from feeding. Based on the results of this study, cage-based tests appear to be a more reliable estimate of repellent activity on skin compared to module-based tests on membrane. However, with knowledge of the effective dose ratios, the module-based tests can be utilized for future repellent testing with infected vectors.

162 Spatial repellents: laboratory, semi-field and field evaluations
Daniel Kline, dan.kline@ars.usda.gov

A spatial repellent is a chemical compound that in the vapor phase can produce repellency at a distance. The major objective is to prevent host-vector contact. A good spatial repellent will significantly reduce the number of vectors in a defined area over a designated period of time. Goals of this study were to find compounds and delivery systems that could protect an individual, a small group of individuals in a confined area (e.g., a tent) or a community of individuals. Candidate compounds and delivery systems were evaluated in a laboratory olfactometer (when appropriate), in a large outdoor enclosed cage or under field conditions (e.g., an area adjacent to a rice field). Both commercial products and novel candidate compounds were evaluated. Some products were insecticides with high vapor action (not considered to be appropriate for olfactometer testing), others were essential oils, and others candidate compounds derived from either natural or synthetic sources. Field studies were conducted against natural populations in Arkansas and Florida.

163 Repellent Efficacy Evaluation: Establishing New Study Designs and Standards
Kevin J. Sweeney, Sweeney.Kevin@epamail.epa.gov

New product develop often requires novel laboratory and field bioassays to evaluate product efficacy. Taken together with requirements associated with human subject testing, this may result in repellent study designs that are not widely accepted or fully validated yet are used by registrants to generate efficacy data for pesticide registration. Examples will be provided for textile, spatial, and skin applied repellent products including an overview on study designs, hypothesis testing, study evaluation and the basis/standard for study success.

164 Comparative behavioral responses of pyrethroid-susceptible and resistant strains of adult *Aedes aegypti* to essential oils from native plants
Theeraphap Chareonviriyaphap, Unchalee Sanguanpong and Sunaiyana Sathantriphop

Behavioral response of mosquitoes to test compounds has long been recognized. In the past, behavioral responses were generally overlooked in national malaria control programs which focused mainly on toxicological study. This study was designed to compare the behavioral responses between susceptible and resistant *Aedes aegypti* against three different essential oils extracted from native plants using an excito-repellency test system. Repellent and irritant actions of three essential oils were evaluated. Preliminary result showed that there were no significant differences in escape responses between susceptible and resistant strains to all oils. Contact response was found to be the primary action on the two compounds, regardless of test populations as DEET was used as a standard reference compound. We conclude that essential oils from these two compounds can be used to protect the bite of susceptible/resistant mosquitoes.

Update on Tick-Borne Diseases in the United States

165 Lyme disease and its control
Alison Hinckley, cue0@cdc.gov

Lyme disease is by far the most common vector-borne disease in the United States. It is transmitted to humans by *Ixodes scapularis* ticks in the Northeast and Midwest and the *Ixodes pacificus* ticks in the West. Lyme disease is most often characterized in early illness by an erythema migrans (EM) rash. Without treatment, the infection disseminates, and patients can develop multiple skin lesions, and/or rheumatologic, cardiac, or neurologic symptoms. Current recommendations for Lyme disease prevention focus on personal protective measures. Personal protection strategies include non-pharmaceutical approaches such as: avoidance of tick habitat, tick checks, and repellent use. Pharmaceutical approaches consist of tick bite prophylaxis and (formerly) vaccination. Although personal protective measures are frequently recommended in endemic areas by healthcare providers and public health officials, data regarding their effectiveness is unclear. Further, their relative acceptance by the at-risk community is important to consider when assessing the utility of specific strategies. In this talk, we will review the available prevention measures and summarize their efficacy and acceptance as reported in published literature. We will also discuss the potential for alternative approaches to tickborne disease prevention.

166 Rocky Mountain spotted fever vector control
Joanna Regan, jregan@cdc.gov

Rocky Mountain spotted fever (RMSF) is a tick-borne disease caused by the intracellular bacterium *Rickettsia rickettsii*. The disease can be rapidly fatal in some cases if not treated in the first 5 days of symptoms. Diagnosis of RMSF can be difficult in these first days when symptoms are often nonspecific and *R. rickettsii* specific antibodies are not likely to be detectable. To further complicate the issue, broad-spectrum antibiotics that are typically used when the cause of sepsis is unknown are not effective against RMSF. The primary vectors in the United States include the

American dog tick (*Dermacentor variabilis*), the Rocky Mountain wood tick (*Dermacentor andersoni*), and the brown dog tick (*Rhipicephalus sanguineus*). The epidemiology of human RMSF varies according to the tick vector present in a given region. Therefore, prevention measures, public health messages and healthcare provider training must be targeted to meet the specific needs of the region. Currently, the areas of the country experiencing the highest incidence rates, as well as the highest case fatality rates from RMSF are several tribal communities in Arizona. The vector for the disease in this region is *Rhipicephalus sanguineus*. This tick feeds primarily on dogs and lives in and around homes, bringing it in frequent contact with humans. The prevention measures currently being undertaken as well as other recent developments related to RMSF will be discussed.

167 Tick-borne *Anaplasmataceae* in the US
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The family *Anaplasmataceae* is comprised of small obligate intracellular bacteria capable of causing disease in a variety of animals. In the US, several of these pathogens exist in life cycles involving tick vectors and mammalian reservoirs in which humans occasionally become infected and serve as dead end hosts. Since first being identified as agents of human disease the number of reported cases worldwide due to these bacteria has continued to grow. In addition, newly identified bacteria in this group have also been shown to cause human illness and previously known *Anaplasmataceae*, once thought to cause disease only in animals, have now been implicated in human disease as well. The diseases caused by these bacteria can range from a mild, self-limiting illness to death. Common symptoms include headache, fever, malaise, and myalgia accompanied by leukopenia and thrombocytopenia. Progression of these diseases can be rapid, so appropriate antibiotic therapy should be initiated as soon as infection is suspected. The incidence of these diseases are limited to the geographic range of the associated vectors and hosts; however these ranges are expanding and human diseases caused by this group of bacteria are being reported in previously non-endemic areas of the US. As these ranges continue to expand and more people come in contact with potentially infected vectors through outdoor activities, the *Anaplasmataceae* will remain a growing public health threat in the US.

168 Babesiosis: an emerging tick-borne infection in the United States
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In the United States, the most common cause of babesiosis in humans is *Babesia microti*. The principle reservoir of *B. microti* is the field mouse and the tick vector is *Ixodes scapularis*. Like the agents that cause malaria, *B. microti* infects red blood cells. Clinical manifestations vary from asymptomatic infection to a life-threatening febrile illness with hemolytic anemia, thrombocytopenia and abnormal liver function tests. Older patients, patients without a spleen and patients who are immunocompromised are at greater risk for more severe illness. *B. microti* can also be transmitted by blood transfusion and is currently the most common cause of blood transfusion-transmitted infection in the United States in which the donor and recipient can be linked. Certain combination antibiotic regimens are effective, but occasionally drug resistance associated with treatment failure has developed during therapy in highly immunocompromised patients.

169 Tick-borne viruses
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Tick-borne viruses causing human illness in the United States include Colorado tick fever virus and Powassan virus. The recently described Heartland virus also may be transmitted by ticks. Colorado tick fever virus is a coltivirus that is transmitted by *Dermacentor andersoni* in the Rocky Mountain region. It causes a febrile illness and is most commonly reported among adult males. The numbers of reported Colorado tick fever virus disease cases have remained relatively constant during the past decade; however, the disease is likely under-recognized and under-reported. Powassan virus is a flavivirus that is transmitted by *Ixodes* spp. ticks. The most commonly recognized clinical syndromes are meningitis and encephalitis. The numbers and demographics of Powassan virus disease cases have changed in the past decade with cases being reported from new locations in mid-Atlantic and north central states and more cases being reported among older adults. Heartland virus, a phlebovirus, also may be tick-transmitted. The virus caused a febrile illness in 2 males in Missouri. Both reported tick exposures prior to their illness. Studies are ongoing to determine the mode of transmission for this virus. To reduce their risk of becoming infected with tick-borne viruses, persons who may come in contact with ticks should be targeted for education about prevention measures, such as using insect repellant, wearing protective clothing, and landscaping their yards to exclude ticks.

GIS/GPS

170 Heads up live mapping solution for ground ULV driver navigation
Ron J. Montgomery, rmontgomery@vdci.net

This presentation will provide a low cost solution for ULV driver navigation utilizing a real-time live mapping program available at many local retailers.

171 Operational success: Pasco County Mosquito Control District's unique use of Mapvision®
Aaron Lloyd, alloyd@pascomosquito.org

In 2010, Pasco County Mosquito Control District (PCMCD) partnered with Leading Edge to develop a centralized web-based GIS mapping system to operate integrated mosquito control efforts in Pasco County, FL. A final

customized product was launched within the district in 2012 as a standalone system coordinating all aspects of PCMCD mosquito control operations. A brief overview of how the mapping system is utilized within PCMCD to coordinate all operational duties will be discussed. In addition, specifics of MapVision® use regarding adulticiding treatment missions will be reported.

172 Operational success: enhancing control with Mapvision® at Pasco County Mosquito Control District
Michael Greer, mgreer@pascomosquito.org and Jack French

The most efficient time to control mosquitoes is in the larval stage. The graphical web based system, MapVision® allows employees to enter inspections and treatments in real or near real time. Essential points of interest show up as icons on the map, which facilitates a visual and intuitive means to both enter data and find useful information. Mosquito habitat targeted for aerial larviciding can be mapped through existing polygons or new polygons can be drawn at the time of inspection. Most data in MapVision® is accessible to supervisors with queries output in spreadsheet format. Customized reports are available for quick assessment and FDACS forms are automatically filled in with the required information.

173 Simplifying GIS for managing mosquito control operations
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Effectively managing a mosquito control operation requires data management tools and skills. A GIS provides enhanced visual analysis and data management software tools that can simplify the management of mosquito control operations. GIS software use becomes more powerful, yet simple, by employing custom software tools designed specifically for creating and maintaining data critical to managing mosquito control operations. This presentation will discuss the use of GIS software tools used in map production, database management, Microsoft Office software integration, and managing data and maps using online resources. In addition, this presentation will discuss 2 GIS software applications, designed and built specifically for mosquito control operations that simplify GIS software use. Sentinel™ GIS is a desktop-based GIS software solution, built upon the Esri ArcGIS for Desktop platform, which simplifies the use of GIS for mosquito control operations. FieldSeeker™ GIS is a server-based GIS software solution, built upon the Esri ArcGIS for Server platform, which simplifies the creation and maintenance of data used for managing mosquito control operations and extends that functionality to the network and internet. This presentation will highlight the advantages of a Desktop PC-based GIS solution and the advantages of a server-based GIS solution.

174 Mapping mosquito control online: the use of a free ArcGIS online personal account to create, store, and manage maps, apps, and data
Jodi Holeman, jholeman@mosquitobuzz.net and Steve Mulligan

In 2012, ESRI released ArcGIS Online, an open platform for maps and geographic information. By creating a free personal account or connecting an existing ESRI global account the end user has the ability to create, store and manage maps and associated data with some limitations. This presentation will outline some of the listed capabilities as well as the limitations of using a free personal account and its applicability to mosquito control.

175 Characterization of the *Anopheles minimus* complex in Thailand
Monthathip Kongmee, monthathipk@yahoo.com, Nicole L. Achee, Penny Masuoka, Pote Aimpun, Kriangkrai Lerdthusnee and Theeraphap Chareonviriyaphap

Anopheles minimus complex plays a significant role in malaria transmission in Thailand. At least two species within this complex are found in Thailand, especially along the Thai-Myanmar border. The objective of this study was to characterize the environmental variables that are associated with high-density oviposition sites of *An. harrisoni* and *An. minimus* to identify predictors of species-specific habitat distribution. A larval survey was carried out in 2 oviposition sites in 2 villages of Kanchanaburi Province, western Thailand during a 2-yr period. Two different ecological habitats of the Minimus Complex from Bong Ti Noy (BTN) and Pu Teuy (PT) were characterized using geographical information system (GIS) and the remote sensing technology in combination with field data. *Anopheles harrisoni* existed at higher density habitats in PT than in BTN, although PT had less *An. minimus* than BTN. In BTN, *An. minimus* was associated with pastureland cover with the highest density habitats being present at short distances from a human habitation (< 180 m). While in PT, *An. harrisoni* was associated with forestland cover with high density habitats being present at further distances from inhabited houses (> 320 m). Species density differences existing between the two study sites indicate that surrounding land cover may be associated with species-specific productive larval habitats.

176 Development of new geo-spatial database software for Maricopa County Environmental Services Vector Control Division
Kirk Smith, ksmith@mail.maricopa.gov, John Townsend and Daniel Damian

Since 2005, our division has utilized the database known as the Vector Control Management System to handle our day-to-day operations. However, since our work load has increased and the software is no longer supported, we decided to look elsewhere for a system that would provide services tailored to our needs. In 2012, we had > 9,000 customer complaints, set over 16,000 surveillance traps, tested 6,000 samples for presence of West Nile virus and made over 10,000 pesticide applications. We approached companies providing existing programs and found that these would not fulfill our demands nor sufficiently handle the workload. With over 73,000 existing locations in the database, we approached Environmental Systems Research Institute, Inc. and with a collaborative effort have

developed a GIS/GPS based system that operates both a desktop application and mobile application via laptop computers. We will discuss the development and demonstrate the useful applications of this new program.

177 Using GIS, spatial analysis and mosquito biology to delineate spray zones for adult mosquito control in New York City

Liyang Zhou, lzhou@health.nyc.gov and Waheed I. Bajwa

Since its discovery in 1999, West Nile virus (WNV) has found a permanent home in New York City (NYC). That year NYC Health Department identified 47 human infections of WNV from all over the city. During the next 10 yr (2000 - 2009), the disease incidence was relatively stable (but varied by season) with an average of 12.5 neuroinvasive cases per year. For the last 3 yr (2010 - 2012), we observed an average of 23 neuroinvasive cases per year in the city. Factors contributing to the higher incidence in the recent past are not well understood but are likely related to climate and ecology. In a non-epidemic year, ground larviciding in the residential areas, parks and cemeteries, and aerial larviciding in marshlands produce good results for WNV control in NYC. During outbreaks, a well-timed adult mosquito control in the "hot spot" areas has been a worthwhile option to reduce the WNV transmission risk to humans and other hosts. In this presentation, we will discuss combined application of geographic information system (GIS), spatial analysis and mosquito biology in determining at-risk areas of WNV transmission and delineating treatment-zones for adulticiding in residential and non-residential areas of NYC.

178 Mapping mosquito control: GIS utility and implementation

Jodi Holeman, jholeman@mosquitobuzz.net and Steve Mulligan

The amount of data a district collects in association with their control operations and how that data is stored varies between districts. Seven years ago the Consolidated Mosquito Abatement District transitioned to storing their data electronically in an integrated geographic information system (GIS).The use of GIS as the base platform for the system was appropriate given the spatial nature of mosquito control. The system was initially designed to meet the data storage needs of the District and was a significant improvement from the paper file records historically used. Despite the improvement, we quickly realized we were not capturing the information we needed to answer many of the questions that arise in a control operation. In the past 7 years, the District's geodatabase has morphed into various iterations that allow for greater functionality beyond just storage. This presentation demonstrates our current approach for managing and mapping information collected in the field and our efforts to better answer some of the questions that tend to arise from that information.

179 Airborne imaging spectroscopy for mapping urban "green pools"

David R. Thompson, david.r.thompson@jpl.nasa.gov, Manuel de la Torre Juárez, Christopher M. Barker, Jodi Holeman, Sarah Lundeen, Steve Mulligan, Thomas H Painter, Erika Podest and Felix Seidel

Evidence suggests that unmaintained residential swimming pools - "green pools" - provide habitat for mosquitoes that transmit West Nile virus. High-resolution remote sensing data can benefit vector control efforts by resolving these pools and flagging specific households for direct intervention. Several studies have demonstrated pool detection in remote imagery. However, to our knowledge no quantitative link has been established between remotely sensed pool color and the probability that the pool contains mosquitoes. This work provides the first such link with the help of airborne imaging spectroscopy. Imaging spectrometers such as the Airborne Visible Infrared Imaging Spectrometer (AVIRIS) typically measure reflected light over large areas in wavelengths from 370 nm to 2500 nm. The full spectrum reflectance data permits a suite of powerful approaches for pool detection and classification. This work examines an AVIRIS overflight of Fresno, CA in the fall of 2011. First, we demonstrate a matched filtering detection strategy to identify pools. Next, we correlate the AVIRIS data with concurrent ground inspections by the Consolidated Mosquito Abatement District. We construct models describing the relationship between pools' status as mosquito sources and observable spectral signatures of stagnant water (specifically chlorophyll due to algae accumulation). This approach predicts the presence of mosquitoes with over 90% accuracy for this dataset.

***Aedes albopictus*: Global Challenge for Surveillance and Sustaining Management**

180 Surveillance and management of *Aedes albopictus* in China

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Both *Aedes aegypti* and *Ae. albopictus* exist in China, while *Ae. albopictus* has a widely geographic distribution with southern areas below latitude 30 degree N. Unplanned urban growth, increasing urban construction, migrant population, with deficiencies in water supply and urban cleaning, favour the proliferation of the vector mosquitoes, and result in big challenges in dengue prevention and control. There is systematic surveillance for dengue and its vector all through the China. Compared to the 1980s and 1990s, the incidence rate was mitigated in 2000-2010. Dengue outbreaks have shifted to the relatively northern regions such as Cixi and Jinhua in Zhejiang province. According to the Global Strategic Framework for Integrated Vector Management published by WHO in 2004, China has instituted sustainable dengue control strategy with close monitoring, accompanied by control of vector mosquito population, high vigilance for imported cases, improvement of diagnosis and treatment, enhancing the cooperation among the different sectors, and mobilizing the social effort to combat the epidemic.

181 Ovitrap surveillance of dengue vectors and their larval habitats in dengue hot spots in Malaysia
Abu Hassan B. Ahmad, aahassan@usm.my, NurAida Hashim and Hamady Dieng

An ovitrap study was carried out to determine the field population abundance of dengue vectors, *Aedes albopictus* and *Ae. aegypti* in 3 dengue hotspots in Penang from February 2008 to March 2010. Thirty ovitraps were placed in each study area and were collected weekly. The urban squatter area had the highest total number of eggs and immature throughout sampling period, followed by urban area and suburban area. The amount of eggs collected were higher in the wet season compared to the dry season. The egg population showed a significant correlation with rainfall and relative humidity but not with mean temperature. Fogging activities more effectively reduced *Aedes* populations during the dry season compared to the wet season. *Aedes* immatures were found in a wide range of habitats but were particularly abundant in artificial containers. The most productive containers are buckets, which comprised 14.74% of larvae/pupae positive containers and provided 18.18% of total *Aedes* immature collected. However, in terms of container usage, discarded plastic-made containers were the most commonly found during this study. *Aedes aegypti* and *Ae. albopictus* showed distinct differences in the container size, preferring large and small containers, respectively. Both species were able to colonize indoor and outdoor, but *Ae. albopictus* prefer outdoor containers. Shared oviposition between *Ae. albopictus* and *Ae. aegypti* was very low.

182 Surveillance and management challenges of *Aedes albopictus* in Mexico
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In 1985, *Aedes albopictus* was introduced into the United States through the Houston, TX, port. Four years later they were identified in Matamoros, Mexico, a bordering location around 400 mi from Houston. Since that time, *Ae. albopictus* has dispersed throughout the country reaching southern borders with Guatemala (Tapachula) and Belize. Entomological surveillance started during the 1990s concentrated *Ae. albopictus* movement mostly on three northern states: i.e., Tamaulipas, Nuevo Leon and Coahuila. Unlike the United States, arrival of *Ae. albopictus* into Mexico presented the country with endemic dengue epidemics. In 1995, *Ae. albopictus* were found with dengue virus serotypes 2 and 3 field infections in Reynosa, another Texas border city. Dengue virus was isolated from a male pool indicating vertical transmission. During 2011, a second report of *Ae. albopictus* vertically infected with dengue was found near Monterrey city. Here, populations of *Ae. aegypti* and *Ae. albopictus* share rural and suburban areas, and circulation of dengue viruses are prevalent each year. The epidemiological role of *Ae. albopictus* in Mexico is becoming a worrying situation for health programs. The WNV outbreak in Dallas, TX, may trigger the vectorial competence of *Ae. albopictus* in northern Mexico to become infected with this arbovirus.

183 *Aedes albopictus* in New York City: an evolving problem
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Aedes albopictus or the Asian Tiger is capable of using natural and artificial standing water sources as larval habitats. This attribute helps it establish in urban, suburban and rural areas. In New York City (NYC), its presence was first observed in 2000 when the NYC Health Department established its West Nile virus surveillance program. Because New York City provides favorable habitats and climatic conditions for colonization, *Ae. albopictus* has become well-established in the City. In addition, the so called "urban heat effects" in the large cities (including NYC) favor mosquito growth. In addition to a positive effect on the mosquito biology, higher temperatures increase amplification of arboviruses that cycle between urban mosquitoes and their hosts. *Aedes albopictus* is a weak vector of West Nile virus (in NYC). However, it is a competent vector of dengue and chikungunya in different parts of the world. Each year NYC has imported human cases of dengue. In 2010, 64% cases were potentially viremic upon returning to the city. Recently, an imported human case of chikungunya was also identified in the city. The presence of imported human cases into a densely populated area with established populations of an efficient vector may pose a potential risk of introduction of exotic arboviruses in the city. We have analyzed geo-referenced surveillance data collected in the last 10 years; the results of these analyses will be discussed in this presentation.

184 Insights into the genetic structure of *Aedes albopictus* in the US
Jiawu Xu, sshus22@gmail.com and Dina Fonseca

The Asian tiger mosquito, *Aedes albopictus*, has been expanding across the US for almost 30 years. Original surveys using allozymes and mtDNA sequences suggested a single introduction from temperate source populations. A genetic survey of its current populations across the states has been lacking because of the difficulty of identifying more informative genetic markers in the highly repetitive genome of this species. From newly collected NextGen sequences and published microsatellite loci, we have optimized a panel of highly polymorphic microsatellite loci and examined the temporal and spatial genetic patterns across several populations in the US. While we observed no significant genetic change over time, we found spatial heterogeneity in genetic signature even at local scales. Furthermore, we identified genetic differences between temperate and tropical forms of *Ae. albopictus*, and, surprisingly, we found that both forms coexist in some populations in the southern US. We discuss the observed genetic structure in the context of introduction history and dispersal patterns and highlight the importance of multiple invasions, especially that of different ecological forms.

185 Insecticide resistance status of the Asian tiger mosquito in the US

Sebastien Marcombe, sebastien.marcombe@rutgers.edu, Ary Farajollahi, Sean P. Healy and Dina Fonseca

Aedes albopictus (Asian tiger mosquito or ATM) is an introduced invasive species in the US responsible for a significant proportion of service requests to local mosquito control programs that result in the application of insecticides. However, to date very limited information is available on the insecticide resistance status of US ATM populations. Because of the possible impact of resistance on ATM control operations we implemented the current studies. We focused on the insecticide resistance status of ATM populations from New Jersey, Pennsylvania and Florida. We implemented larval and adult bioassays with a range of insecticides representing classes or types of insecticides with different modes of action currently or historically used in the US for mosquito control. Larval bioassays revealed overall complete susceptibility to most insecticides but we did find some populations with reduced susceptibility to a carbamate. Similarly, most adults tested with WHO tube tests were fully susceptible to the majority of insecticide classes, but, surprisingly, we found evidence of high levels of resistance concentrated in a few populations. To investigate the possible mechanisms involved in resistance such as metabolic-based resistance and target-site resistance, we developed both biochemical and DNA based assays. In light of the results, we will discuss the efficacy of different insecticide classes used for ATM control, and the resistance, or cross resistance patterns in US ATM that may threaten control operations.

186 Cost-benefit analysis of an area-wide pest management program to control the Asian tiger mosquito in New Jersey

Donald Shepard, shepard@brandeis.edu, Yara A. Halasa, Eve Wittenberg, Dina M. Fonseca, Ary Farajollahi, Sean P. Healy, Randy Gaugler, Kristen Bartlett-Healy, Daniel Strickman and Gary G. Clark

Area-wide pest management (AWPM) is recommended to control urban mosquitoes such as *Aedes albopictus* (Asian tiger mosquito) which limit outdoor activities. We conducted a cost-benefit analysis for an AWPM in Mercer and Monmouth counties, New Jersey, as part of a controlled design with matched areas (AWPM vs. control). The evaluation analyzed financial documents, assessed staff time, and surveyed 311 to 396 households annually to assess their "hours lost." These were differences between actual and potential hours of yard and porch activities. The AWPM reduced hours lost by 1.88±4.19 (mean±SEM) hours/week in intervention areas compared to control areas for yard and porch activities, indicating potential program effectiveness (p=.32). This translated to 24.4 h gained over the 13-wk summer. The economic benefits were valued based on respondents' willingness to pay (WTP) for a hypothetical extra hour free of mosquitoes that they could spend in yard or porch activities. Residents' WTP averaged $2/h (range: $1-$3), indicating a monetary valuation from fewer hours lost per resident of $49/yr (range: $24-$73). The average per capita expenditure on AWPM was $30/yr. These figures show a net benefit per resident of $19/yr (range: -$6-$43) and a benefit-cost ratio of 1.63 (range: 0.81-2.44), indicating $1.63 of benefits per $1.00 in costs. The cost-benefit analysis suggests a positive net benefit of the AWPM from residents being able to enjoy more time on yards and porches.

Disaster Response and Vector Control I

187 Vector control policy, procedures and coordination for federal disaster assistance

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In the wake of hurricanes, tornadoes, or flooding events, significant increases in the populations of mosquitoes may occur. Swarms of mosquitoes may represent a severe nuisance to response and recovery workers or a severe health threat to the general public because of the increased risk of mosquito-borne diseases. If a presidential disaster declaration is received for the impacted area, then FEMA's Public Assistance Program may provide federal disaster assistance for vector control activities as per the requirements in FEMA's Policy 9523.Ten eligibility of vector control. Surveillance and mosquito control costs may be reimbursed when several conditions are met, including demonstration of a significant increase in mosquito populations beyond normal levels and the increased risk to public health and safety. For reimbursement, communities must document all activities and coordinate with local, state and federal entities prior to spraying. FEMA works with the state and communities to meet eligibility requirements and facilitates the coordination between federal, state, and local partners, including local and state health departments, the Centers for Disease Control, the US Fish and Wildlife Service, and other federal or state resource agencies as applicable.

188 Military entomological resources for emergency mosquito control

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The Army, Navy, and Air Force all maintain advanced-degree entomologists as part of their medical officer corps with the primary objective of protecting the health of the United States' armed forces. Nonetheless, these military entomological resources can be used to provide support to civil authorities as directed by the Secretary of Defense. The employment of Defense Support to Civil Authorities (DSCA) is relatively complex and involves the orchestration of such factors as the proper request process, funding, and "chain of command" issues, to name a few. Once approved and mobilized, however, these Department of Defense assets can be of significant help to civil authorities when dealing with emergency mosquito control. This paper will address some of the policy issues surrounding the use of DSCA, outline the resources available from the individual military services, and provide examples of their previous role in contingency mosquito control.

189 CDC roles and responsibilities in disaster situations
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Natural disasters, both domestic and foreign, occur with some regularity. When they occur, CDC is often called for help. Responses vary depending on the nature of the event and range from telephone consultation to sending teams of responders to the site. In addition to direct help to those affected by the event, CDC also supports FEMA by providing them consultation during the reimbursement process for vector control matters. Examples of past responses and outcomes will be discussed.

190 Coping with Hurricane Irene - lessons learned by Delaware for FEMA reimbursements
William H. Meredith, William.Meredith@state.de.us

When hurricanes strike Delaware, damages are usually not enough for FEMA to declare an emergency that would make our mosquito control program eligible for cost recovery. In August 2011, Hurricane Irene dumped a foot of rain in 24 hours, along with coastal flooding and wind damage. This led to considerable aerial larviciding and adulticiding over a 3-week period, done without our thinking we'd ever be eligible for cost recovery from FEMA. Storm-related costs consumed about 30% of our annual operational spray budget, putting us into a serious fiscal hole. About a month after the storm's passage and our control work completed, quite surprisingly we were told that storm damages were severe enough to be eligible for cost recovery. We then had to scramble to make contacts with our state public health officials that should have started the day after the storm hit. We also had to compile and analyze a lot of pre-storm vs. post-storm data for mosquito population levels or arbovirus indicators, along with amounts of mosquitocides used. For baseline purposes, we had to provide averages for such metrics for equivalent time periods during 3 non-storm years. Providing this documentation took considerable labor, but fortunately, with the CDC's guidance, we were able to get it done. About 6 months later, we received the maximum 75% allowable cost recovery for our storm-related costs. We're now much more knowledgeable for what to do the next time a hurricane hits.

Larval Control I

191 Larvicidal activity of leaf crude extract from *Ipomea cairica* against dengue and dengue hemorrhagic fever vectors
Wan Fatma Zuharah, wfatma@usm.my, Ahbi Rami Rattanam, Thiagaletchumi Maniam and Nik Fadzly

Viral infection of dengue transmitted by the mosquito of the genus *Aedes* is one of the main health hazards worldwide, especially in tropical and subtropical countries. A natural product from a plant compound is considered as one of the safest methods to control mosquitoes because it is rich in bioactive ingredients. The larvicidal efficacy of leaf crude extract of *Ipomoea cairica* or coast morning glory was evaluated using 24 h larval bioassay against dengue and dengue hemorrhagic fever vectors, *Aedes albopictus* and *Ae. aegypti*. Two different solvents (methanol and acetone) were used to enhance the effectiveness of crude extract and were tested at concentrations ranging from 10 to 450 ppm. Better larvicidal activity with lower concentration 50 (LC50) of *I. cairica* crude extract was observed for *Ae. aegypti* for both solvents; acetone (101.94 ppm) and methanol (114.78 ppm) when compared to *Ae. albopictus*; 105.59 ppm and 122.12 ppm, respectively. Though extraction using acetone solvent had enhanced the best larvicidal activity from *I. cairica* crude extract to control *Ae. albopictus* and *Ae. aegypti*. Results also suggested that *I. cairica* leaf extracted using methanol and acetone solvents have huge potential to be used as an ideal eco-friendly approach in controlling dengue vectors.

192 Ovicidal efficacy of insect growth regulators against *Aedes* and *Culex* mosquitoes and their relationship with water permeability
Anwar L. Bilgrami, anwarbil@rci.rutgers.edu, Devi S. Suman, Yi Wang and Randy Gaugler

Mosquito management is mostly confined to the larval and adult stages. The untargeted egg stage thus helps population resurgence. Recently, insect growth regulators (IGR) drew attention as ovicidal agents. Experiments determined ovicidal efficacy of IGRs i.e., azadirachtin (AZD), diflubenzuron (DFB) and pyriproxyfen (PPF) against freshly laid and embryonated eggs of *Aedes albopictus, Ae. aegypti, Ae. atropalpus* and *Culex pipiens pipiens* and relationship between IGR efficacy and water permeability of freshly laid eggs. Maximum hatching inhibition was recorded in *Ae. albopictus* at 1 ppm of PPF (80.6%), AZD (42.9%) and DFB (35.8%). The efficacy of PPF (47.3%), DFB (25.5%) and AZD (15.7%) was against *Ae. aegypti* eggs. The efficacy of IGR against embryonated eggs was reduced at high concentrations as compared to the freshly laid eggs. The water permeability depended on egg desiccation time, the highest being recorded in *Ae. atropalpus* (5.1 h) followed by *Ae. aegypti* (4.9 h), *Ae. albopictus* (3.9 h) and *Cx. pipiens* (1.7 h). Although, hatching inhibition was independent of egg desiccation, results indicate different effects of IGR and existence of resistance in eggs. When exposed to DFB, larvae hatched unusually from the side of the egg wall. What implication this might have on the efficacy of IGR is yet to be explained. However, varying egg susceptibility and dose dependent efficacy suggests that IGR can play significant role in mosquito control.

193 Field evaluation of an autodissemination station using an insect growth regulator
Devi S. Suman, dssuman@rci.rutgers.edu, Yi Wang, Ary Farajollahi, Gregory Williams, Eric Williges, Jacques C. Bertrand and Randy Gaugler

Aedes albopictus is an anthropophilic outdoor biting mosquito that prefers oviposition in small to medium-sized cryptic habitats. This behavior creates hurdles for conventional chemical control strategies, which may have low

penetration to these habitats. Autodissemination is a management approach in which oviposition-seeking females contaminate themselves with insecticide and then disseminate the chemical to larval habitats. An autodissemination station was tested under field conditions using natural mosquito population. We were interested to know the number of stations required to disseminate bio-effective IGR dose to various numbers of competing oviposition sites in an area. These studies were conducted by creating cryptic habitats in 1 acre treatment plots. For these studies, 2 stations were deployed against 5, 10, and 20 oviposition cups per plots whereas other plots had 10 oviposition cups against 1, 2, and 4 station per plot. The station was also assessed against junkyard and tire piles habitats as these places are the most preferred and major oviposition sites for the Asian tiger mosquito. Autodissemination efficacy of the station was measured as pupal mortality by IGR-bioassay of field samples using 3rd instars under standard laboratory conditions. The initial results showed autodissemination of pyriproxyfen under the field conditions. Results may be helpful to formulate area-wide management approach of Asian tiger mosquito.

194 Bi-phase autodissemination station enhanced transfer of insect growth regulator to mosquito oviposition sites

Yi Wang, ywangs@hotmail.com, Jacques C. Bertrand, Devi S. Suman, Limin Dong and Randy Gaugler

Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), is an urban species that colonizes cryptic small-volume larval habitats and a skip ovipositor. These larval habitats are often difficult to reach by conventional larvicidal applications. We have developed an autodissemination station (ADS) that exploits gravid female oviposition behavior for the transfer of insect growth regulator (IGR) from the station to oviposition sites. Our station consists of a water reservoir that attracts gravid mosquitoes and a transfer chamber that allows mosquitoes to exit through a bi-phase (liquid and solid) treatment system. Bi-phase IGR formulations enhance attachment and persistence to a mosquito and release well into subsequent oviposition sites. In room ($31m^3$) trials with *Ae. albopictus*, 100% emergence inhibition was achieved. In a greenhouse ($88\ m^3$) assay, average performance for each ADS against 6 ovicups is 57 + 11.4% emergence inhibition despite cups in the area of unsuitable condition. The evidence of transfer, mosquito footprints, was visualized with a florescent dye under UV light. The GC/MS and lab bioassay show that amount of active ingredient transferred by individual mosquito was sufficient to cause 100% mortality of the mosquito larvae in a container with 250 ml water. The bi-phase ADS is a promising device to control container mosquitoes.

195 Efficacy and non-target effects of Natular™ 2EC and 30G formulations

Sharon P. Lawler, splawler@ucdavis.edu and Deborah A. Dritz

We tested the efficacy and non-target effects of 2 Natular™ mosquito larvicides, 2EC liquid and G30 granules (Clarke Inc, Roselle, Il). Natular products are certified for organic agriculture and are made with spinosyn toxins derived from bacteria. Aquatic target species include mosquitoes and chironomid midge larvae. Tests occurred in 15 pond mesocosms set in a seasonal wetland. Five tanks were controls, 5 received 2EC at 2.5 oz/acre and 5 received G30 at 13 lb/acre. Two screened sentinel cages were added to each tank, containing 10 mayfly larvae as non-targets or 20 2nd instar *Culex tarsalis* larvae as targets. Survival was monitored at 24, 48 and 72 h post-treatment. Thereafter, sentinels were replaced weekly and monitored weekly for survival. Chironomid midges and uncaged non-targets were monitored weekly using standardized hand-net samples. The study lasted 42 d after treatment. Natular showed efficacy against mosquitoes and chironomids for at least 28 d. There was increased survival of mosquito sentinels and chironomid populations near the end of the experiment in 2EC mesocosms but continued high mortality in G30 mesocosms compared with controls. Non-target insects were also affected, but sentinel mayfly survival converged with controls by day 21 after treatment with both liquid and granular formulations.

196 Effects of spinosad on oviposition choice of *Aedes albopictus*

Fatima Cheema, facheema@eden.rutgers.edu, Scott C. Crans and Randy Gaugler

Recent dengue outbreaks support area wide efforts to control the vector transmitting diseases. Integrative pest management programs often include a variety of destructive techniques to interrupt a mosquito's life cycle. Spinosad, a biologically derived pesticide, has recently been formulated as a widely available larvicide. It exhibits wide margins of safety for humans and has a favorable environmental profile (US EPA 2007). The aim of the study was to test the behavioral impact of spinosad on the oviposition preference of gravid female *Aedes albopictus*. The data was analyzed using a paired t-test. The results indicated a slight preference for the control solutions over the spinosad-treated solutions (LC 50), characterizing the insecticide as a mild repellant. Testing oviposition choice at different concentrations of spinosad can further enhance the study.

197 Natular™ use in catch basins in Florida

James F. Clauson, jamesclauson@comcast.net and Dale Martin

Beach Mosquito Control District has over 5,000 catch basins, many of which are prolific habitat for mosquitoes. In an effort to rotate larvicides and increase effectiveness of control measures, Beach Mosquito Control District conducted a field trial with extended release Natular™ XRT Tablets. The study was conducted during the spring/summer of 2012.

198 Efficacy of a slow-release formulation of novaluron in extended field trials

Paddy McManus, pmcmanus@uoguelph.ca, Lindsay Furtado, Rob Dupree and J. Christopher Hall

Mosquitoes pose a serious risk to public health around the globe as vectors of human diseases such as the West Nile virus, malaria and dengue fever. Consequently, it is extremely important to control mosquito populations especially at their larval habitats, i.e., standing water. A number of pesticides including novaluron, a member of the benzoylphenyl urea chemical family, are effective at controlling mosquitoes; however, in many cases reapplication is required to provide effective season-long control. A new slow-release, wax-based formulation of novaluron has been developed by Tumaini Controlled Release Technologies Inc. to regulate the release of the active ingredient in standing water allowing for a prolonged mosquito control period. This novaluron formulation was added to a set of 12,000-L aquatic mesocosms to measure its efficacy against mosquito larvae and rate of degradation. Mesocosms were divided into 5 groups: novaluron at 2 different levels of application, each with and without added organic sediment and a control. The concentration of released novaluron was measured for 318 d (including over-wintering) and was compared with *Aedes aegypti* bioassay results. Regardless of the presence of added organic sediment the high concentration of novaluron provided at least 95% inhibition of emergence 318 days after application.

***Aedes albopictus*: Lessons Learned from Area-wide Management**

199 The USDA-ARS area-wide project for management of the Asian tiger mosquito, *Aedes albopictus*

Daniel Kline, dan.kline@ars.usda.gov and Gary G. Clark

The Asian tiger mosquito, *Aedes albopictus*, is among the most invasive species in the world. Established in the US since 1985, this species now infests 30 states and continues to spread internationally. Concerned public health officials recognize this species as an important vector of chikungunya and dengue viruses and it is regarded as the most significant nuisance mosquito across its range. In response to this situation, the USDA Agricultural Research Service obtained funding in 2007 to establish an "area-wide" project focused on the management and control of this species. The project was designed to be a unique federal, state, local collaboration based at the Center for Vector Biology at Rutgers University. Carefully planned and implemented research projects with Mercer and Monmouth counties have served to evaluate and develop enhanced methods for surveillance and control of this species. Research has included exploration of the role of educational efforts in controlling mosquitoes, definition of urban production sources in urban areas, and efficacy of different adult control measures. A novel aspect of this project was the partnership with economists at Brandeis University in Waltham, MA, to study the willingness to pay for enhanced mosquito control, among other economic issues. Results and "lessons learned" during this 5-year project are presented in this symposium along with initial results of project extension to several US states.

200 Lessons learned about educating towards mosquito control

Kristen Bartlett-Healy, krisbh@verizon.net

The goal of this study was to evaluate the effectiveness of a public educational campaign to reduce backyard mosquito-larval habitats. Three communities each, within 2 New Jersey counties, were randomly selected to receive (1) both education and mosquito control, (2) education only, and (3) no education or mosquito control. In year one, 4 separate educational events included a 5-day elementary school curriculum in the spring, and 3 door–to-door distributions of educational brochures. In year two, we implemented an active educational program, involving community peer educators, tire pick up days, and trashcan drilling days. Before and after each educational event in both years, the numbers of mosquito-larval container habitats were counted in 50 randomly selected homes per study area. Although we saw reductions in container habitats in year one in sites receiving education, they were not significantly different from the control. During year 2, we saw significant reductions in container habitats following active educational programs. Our results suggest that traditional passive means of public education, which were often considered the gold standard for mosquito control programs, are not sufficient to motivate residents to reduce backyard mosquito-larval habitats.

201 Effort to suppress *Aedes albopictus* populations using a hot spot approach

Isik Unlu, iunlu@mercercounty.org, Ary Farajollahi, Nick Indelicato, Taryn N. Crepeau, Sean P. Healy, Randy Gaugler and Dina Fonseca

Aedes albopictus (Skuse) is recognized as a major pest because of its aggressive anthropophilic host preference and daytime biting behavior. In addition, it is an effective vector of dengue and chikungunya viruses and is susceptible to many other arboviruses, making this species also a public health concern. Therefore, where it has become established (30 US states) there is a growing demand for an efficient and cost-effective panel of control strategies for *Ae. albopictus*. Although source reduction has been shown to be effective at reducing populations, thousands of back yards need to be inspected during door-to-door source reduction campaigns, a very labor intensive and not cost effective technique. We tested if localized "hot-spot" source reduction integrated into a control program to eliminate high adult mosquito populations worked. Hot spot treatments included: removal of containers, larvicide applications, cutting over-grown vegetation in abandoned parcels, barrier spraying, and education. Field crews responded to 8 hot spots between 6 June and 20 July 2011 (not more than 2 hot spots per week), and managed to keep the *Ae. albopictus* populations below set threshold of 5 (male and female; using BGS traps for surveillance) with only hot spot treatments in 1,251 parcels in Trenton, NJ.

202 *Aedes albopictus* in northeastern suburban coastal communities

Sean P. Healy, shealy@co.monmouth.nj.us, Ary Farajollahi, Taryn N. Crepeau, Isik Unlu, Randy Gaugler and Dina Fonseca

Our objective over the last 5 years has been to develop an effective and operationally feasible management strategy for the area-wide control of *Aedes albopictus*. Since 2008, we have investigated educational approaches, surveillance techniques, pesticides, application equipment, and application strategies and evaluated them as part of the area-wide control program in suburban coastal communities in Monmouth County, New Jersey. This presentation will summarize the knowledge gained on the biology of *Ae. albopictus* in this suburban habitat, in contrast to more urban environments, and its impact on all aspects of implementing a successful area-wide control program.

203 Killing tigers in a concrete jungle: effectiveness of a nighttime ULV adulticide against diurnal *Aedes albopictus*

Ary Farajollahi, afarajollahi@mercercounty.org, Sean P. Healy, Isik Unlu, Taryn N. Crepeau, Dina M. Fonseca, Randy Gaugler, Dan Strickman and Gary G. Clark

Aedes albopictus, the Asian tiger mosquito, continues expanding its geographic range and involvement in mosquito-borne diseases such as chikungunya and dengue. Vector control programs rarely attempt to suppress this diurnal species with an ultra-low volume (ULV) adulticide because for maximum efficacy, applications are conducted at night. During 2009-2011, we performed experimental nighttime applications of a novel adulticide (DUET®) against field populations of *Ae. albopictus* within an urban site composed of approximately 1,000 parcels (home and yard) in northeastern USA. Dual applications at mid-label rate of the adulticide spaced 1 or 2 days apart accomplished significantly higher control (85.0 ± 5.4% average reduction) than single full rate applications (73.0 ± 5.4%). Our results demonstrate that nighttime ULV adulticiding is effective in reducing *Ae. albopictus* abundance and highlight its potential for use as part of integrated mosquito management programs and during disease epidemics when reducing human illness is of paramount importance.

204 *Aedes albopictus* control in southern Louisiana

Dawn Wesson, wesson@tulane.edu

Aedes albopictus control activities performed in 3 southern Louisiana parishes during the summer of 2012 will be described. A variety of control operations, include those targeting adult and larval life stages, were performed in Orleans, St. Tammany and Calcasieu parishes. Methods included aerial and truck mounted ULV applications; surveillance relied on oviposition trapping and experimental larval mortality assessments, as well as adult mosquito surveillance by BG Sentinel trapping and human landing counts. The outcome of these efforts will yield information important to vector control activities in similar locales around the world.

205 *Aedes albopictus* control in Volusia County, Florida

James McNelly, jmcnelly@volusia.org

Located in central Florida, Volusia County Mosquito Control (VCMC) was founded 75 years ago to contend with salt marsh mosquito production. Container mosquito production (*Aedes albopictus*) and the resulting service requests, and the surveillance and control efforts of VCMC personnel now rival salt marsh related responsibilities. As part of a nationwide, USDA-ARS effort to evaluate and invigorate *Ae. albopictus* surveillance and control practices, VCMC initiated a season-long program directed at *Ae. albopictus*. The program was based upon recent applied research and the recommendations of the USDA-ARS Area-wide Asian Tiger Mosquito Management Project Team. This 2012 program is the first such structured and sustained program in Volusia County and was supported in part through USDA-ARS and Rutgers Center for Vector Biology funding. The presentation will discuss the use of BG Sentinel traps to monitor adult populations at 2 sites over the 2012 season, efforts related to larval surveillance and control in backyards and ULV adulticide applications. The future direction of VCMC *Ae. albopictus* efforts will be discussed.

206 *Aedes albopictus* control in the oldest city (St. Augustine, FL) in the US

Rudy Xue, xueamcd@gmail.com

Aedes albopictus is one of major pest and vector mosquitoes. The mosquitoes oviposit in many kinds of containers and bromeliad flowers around residential areas. Control of the mosquitoes in the oldest city is always challenging. Anastasia Mosquito Control District, St. Augustine, FL, conducts regular inspection (larval presence, landing rate counts, and trapping by BGS traps) and control of the mosquitoes by using source reduction (collecting tires & empting containers), thermal fogging of adult mosquito resting habitats (vegetation) with ANVIL 10+10 or DUET, conducting barrier treatment with bifenthrin, or spraying attractive toxic sugar baits on vegetation against adult *Ae. albopictus*. The application methods and techniques are depending on locations, vegetation types and distribution, and mosquito population. The integrated mosquito management is suitable to control of the mosquitoes in the oldest city.

207 Area-wide management of the Asian tiger mosquito: summary of lessons learned

Dina M. Fonseca, dinafons@rci.rutgers.edu and Gary G. Clark

Aedes albopictus, the Asian tiger mosquito, is the principal vector of chikungunya and a critical vector of dengue viruses. This daytime biting pest is now distributed over much of the eastern quadrant of the continental US all the way north to coastal New York, and often causes the majority of service requests from urban and suburban residents

in New Jersey (NJ) and many other states and nations where it has spread. Our objective was to develop an effective strategy for the area-wide control of *Ae. albopictus*, while demonstrating the public health importance and socio-economic benefits of the area-wide control approach. The project was initiated in the spring of 2008 in 2 counties in NJ, and after 5 years we have tested multiple control and educational interventions, as well as gauged public interest, public participation, and overall costs. We have also examined baseline levels of insecticide resistance and the genetic homogeneity of US populations of *Ae. albopictus*, and partnered with mosquito control programs in other affected states to extend our findings. We will highlight major findings, lessons learned, and some of what still needs to be done for the development of integrated management strategies for the Asian tiger mosquito.

Disaster Response and Vector Control II

208 Tips for a successful FEMA application - from a county mosquito control agency
Christine Musa, cmusa@warrencountymosquito.org and Jennifer Gruener

Warren County, NJ, experienced a year of high precipitation and increasing West Nile virus activity as we entered into August 2011. Then rainfall from Hurricane Irene and remnants of Tropical Storm Lee created massive flooding in the northern portions of the county. These storms resulted in large populations of floodwater mosquitoes, which affected residents and emergency crews working to clean up and make road/bridge repairs following the storms. The Warren County Mosquito Commission had to utilize $30,000.00 of funding above and beyond what its budget appropriation was for the year in response to these back-to-back storm events. The success in securing reimbursement from FEMA for the costs directly associated with these disasters was a result of detailed record keeping, persistent communication with FEMA representatives, and the ability to clearly show how extraordinary our actions were post disasters.

209 Securing a select agent during Hurricane Irene 2011
Karen Hedstrom, khedstrom@co.cape-may.nj.us, Diane McNelly and Peter J. Bosak

The Cape May County Department of Mosquito Control is the only county mosquito control agency in New Jersey that maintains a Biological Safety Level 3 (BSL3) laboratory. The select agent (SA) eastern equine encephalitis virus (EEEV) was used as a polymerase chain reaction (PCR) positive control in the detection of EEEV in mosquitoes and is regulated by the Centers for Disease Control and Prevention, Division of Select Agent and Toxins. These regulations dictate the handling of a SA in the event of a natural disaster such as we experienced with the threat from Hurricane Irene in August 2011. We will present the events leading up to Hurricane Irene and the protocols followed in securing the SA.

Vector Studies/Surveillance

210 Surveillance and control of *Aedes albopictus* in the San Gabriel Valley, Los Angeles County, California
Teresa Sorvillo, tsorvillo@sgvmosquito.org, Kelly Middleton, Bryan Sorvillo, Marta Tanaka and Kenn Fujioka

In September 2011, *Aedes albopictus* was discovered for the first time in Los Angeles County, CA, since 2001. A significant population has since been uncovered, primarily within approximately 2486.39 ha in the City of El Monte, CA. Additional specimens were collected from the neighboring cities of Duarte and Arcadia, though population densities in these areas are yet to be determined. Our vision for 2012 was to determine the extent of the infestation while working toward the ultimate goal of eradication. Here we provide an update on the status of the infestation and outline the various strategies employed during our first full year of *Ae. albopictus* surveillance and control.

211 Under the radar: is *Aedes aegypti* resurging in Duval County, Florida?
Jennifer Wright, jennifer.wright@med.navy.mil, Ryan Larson, Peter Obenauer, Craig Stoops and Marah Clark

The first record of *Aedes albopictus* (Skuse) in the state of Florida occurred in 1986 at a waste tire site in Jacksonville (O'Meara et al. 1992). Over the past 25 years, the once dominant urban container species, *Ae. aegypti* (L.) has become almost completely extirpated throughout much of northeast Florida (O'Meara et al. 1993). Our study aimed to characterize the current *Ae. albopictus* and *Ae. aegypti* population dynamics in Jacksonville, FL, and determine possible environmental factors influencing these populations. We monitored both populations using CO_2-baited BG-Sentinel and oviposition traps at 10 suburban sites for 12 consecutive weeks from June to September 2012. Nearly 14,000 mosquitoes were captured and approximately 2,600 eggs collected over the duration of the study. F1 generation *Ae. aegypti* were tested for resistance to Dibrom and 30-30 permethrin which are commonly used by Jacksonville Mosquito Control District. Our study suggest that the *Ae. aegypti* population has dramatically increased and has become the dominant species in areas nearest the Ortega and St. Johns rivers and may be spreading geographically. The views expressed in this abstract are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the US Government.

212 Sand flies (Diptera: Psychodidae) of Liberia
Peter J. Obenauer, obenauerp001@yahoo.com, Shabaan Elhossary, Noha Watany, Joseph Diclaro, Craig Stoops, Fatorma Bolay and Desmond Foley

Little is known about the sand fly fauna of Liberia. In Africa, phlebotomine sand flies are the main vectors of *Leishmania* parasites, and leishmaniasis is endemic to North, Central and East Horn of Africa; leishmaniasis is also endemic in parts of West Africa but remains less recognized, often considered as one of the most under reported vector-borne diseases in the region. This is a result, in part, of a lack of information on species distribution. To

identify potential leishmaniasis vectors and their distribution, we conducted a sand fly surveillance study from January 2011 to July 2012. Sand flies were collected in 5 counties of Liberia every 8 wk for 5 consecutive nights using 3 types of light traps. A total of 2,804 sand flies, representing 21 species from 2 genera were collected; the majority were identified as *Sergentomyia squamipleuris, S. dissimillima, S. simillima, S. africana,* and *S. distincta*. The seasonal distribution of these species along with other potential vectors of leishmaniasis will be discussed.

213 Hourly flight times for host seeking female mosquitoes in southwest Florida
James H. Burgess, burgess@lcmcd.org, Tom Miller, Wayne Gale and Jonathan Hornby

This is a compilation of data from host seeking mosquitoes and their flight times. A time trap configuration was set up in a freshwater habitat and a saltmarsh habitat. The configuration was 2 programmable bottle collection devices with a CDC light trap mounted on top of each. The collection device is a rotating wheel that consists of a programmable timer with 8 collection bottles. Only 7 were used for collection and 1 as a stopping station. Collections were made 1 day a wk, for 52 wk. On the night of collection, the first device would turn on its CDC light trap for 1 h, and then rotate to next bottle for 1 h and so on for a total of 7 h. At which time the second device would turn on and continue in this manner, till a 14-h time period was covered. The first collection device began at 5:00 p.m. and the last collection hour 12:00 a.m. The second device would start at 1:00 a.m. and the last collection hour was 7:00 a.m. These were baited with 4-5 lb of dry ice. The collection bottles were collected shortly after 8:00 a.m. With the exception of a few down times for maintenance and hurricanes, these traps have been run every week for the past 3 yr. The data indicates dusk and dawn are not the prime flight times as thought.

214 Effectiveness of TrapTech® mosquito lure added to CO_2-baited CDC miniature traps with and without light and effectiveness when added to CO_2-baited MMX traps without light in capturing *Aedes japonicus japonicus*
John F. Anderson, john.f.anderson@ct.gov, Francis J. Ferrandino, Robert Bedoukian and Susan McKnight

We recently reported that the CDC miniature light trap baited with CO_2 and TrapTech® Mosquito Lure (250 mg. R-1-octen-3-ol and 1900 mg. ammonium bicarbonate) (Bedoukian Research, Inc.) averaged 567 *Aedes japonicus japonicus* per trap per night, 28 times as many specimens as were captured in CDC miniature light traps baited with CO_2 only (J Am Mosq Control Assoc (2012) 28:184-191). In experiments conducted in 2012, the CDC miniature light trap baited with CO_2 and TrapTech® Mosquito Lure captured 620 specimens of *Ae. japonicus japonicus* per night per trap compared to 20 specimens in CDC miniature light traps baited with CO_2 only. CDC traps without light but baited with CO_2 and TrapTech® Mosquito Lure averaged 10 specimens per trap per night. MMX traps without light baited with CO_2 and TrapTech® Mosquito Lure averaged 320 specimens per trap per night. Possible reasons for the disparity in the collection results of *Ae. japonicus japonicus* between the MMX traps without light baited with CO_2 and TrapTech® Mosquito Lure and the CDC miniature traps with and without light but baited with CO_2 and TrapTech® Mosquito Lure will be discussed.

215 Advantages of using multiple rotating traps and the time of activity for *Aedes sierrensis*, *Ae. dorsalis*, and *Culex tarsalis*
Banugopan Kesavaraju, banu@slcmad.org and Sammie L. Dickson

Time of activity for mosquitoes is important to understand the optimal time to spray insecticides to control them. Rotating traps have been used by many mosquito abatement programs to understand the time of activity of their target species. Most often only one rotating trap is used to gather this data. Mosquito abundances and time of activity can be influenced by several environmental factors, and in this presentation we discuss the advantages of using multiple rotating traps. We used multiple rotating traps to understand the time of activity for the common mosquito species within the Salt Lake City area and we discuss those results in this presentation.

216 Inadvertent creation of a species-specific trap by adaptation of a novel Australian design
Donald Shroyer, d.shroyer@irmosquito2.org, Scott A. Ritchie and Andrew van den Hurk

Researchers in Australia have recently developed a "passive" (i.e., non-powered) carbon dioxide-baited mosquito trap for use in arbovirus surveillance. This unusual and seemingly improbable trap design can be readily constructed and modified using locally available materials. After field evaluations of the prototype traps in Indian River County, Florida an attempt was made to create a more rugged version of this trap for continued use in our District's surveillance program. Latin square testing protocols were used to compare collections of the new trap with dry ice-baited CDC light traps and lard can traps (the latter also "passive" in nature). Collections in our passive trap variant were far less diverse than other trap types, including the original Australian prototype traps, and almost exclusively collected *Culex nigripalpus* females. Though not appropriate as a general mosquito surveillance trap, our modified trap is useful in trapping large numbers of pristine, host-seeking *Culex nigripalpus* for experimental use, dissection or arbovirus isolation.

217 Motorized resting boxes: adding a new look on an old collection idea
Dale Martin, martin_dale@comcast.net

Resting Boxes have been a part of Beach Mosquito Control District's surveillance since 2002. In the September 2011, Journal of the American Mosquito Control Association, there was an article under Scientific Note: The Centers for Disease Control and Prevention Resting Trap: A Novel device for Collecting Resting Mosquitoes. After reading the article, I talked with Beach Mosquito Control director James Clauson and chief maintenance person Lee Dukes. We undertook a project of motorizing the resting boxes for collections. Three of the motorized boxes were place in the

field for trial this season, 2012. I will briefly show and describe the making of motorize resting boxes and some of the ways I'm hoping it will improve the surveillance here at Beach Mosquito Control District, Panama City Beach, FL.

Sugar Feeding Behavior of Biting Flies and Attractive Targeted Sugar Baits (ATSB)

218 Prospects for controlling phlebotomine sandflies with insecticidal baits
Graham B White and A. Burkett

Only recently has it been recognized that both sexes of sandfly adults normally ingest sugars from plants, apart from periodic blood-feeding by female sandflies. Sandfly males and females apparently bite plants to obtain sap from stems and leaves, as well as sugar-feeding from fruits, flowers and extra-floral nectaries. Hence, insecticidal sugar baits may be useful against sandflies as well as mosquitoes. We present highlights of projects supported by the Deployed War-fighter Protection program, administered by the Armed Forces Pest Management Board . Studies presented: Muller & Galili preparing and applying attractive toxic sugar baits (ATSBs) for reduction of sandfly survival and risks of vectoring leishmaniases; Orshan & Zollner evaluating environmental factors affecting ATSBs; Faiman & Warburg on sandfly behavior and ecology influencing ATSB applications; Foil & Mascari targeting sandfly larvae with rodent feed-through insecticidal baits that may also impact female sandflies systemically via bloodmeals; and Poché et al. for commercializing Kaput™ rodent insecticidal bait with EPA approval. We outline potential roles of these new products for integrated vector management of phlebotomine sandflies.

219 Recent attractive toxic sugar bait ATSB trials in St. Augustine
Edita E. Revay, Rui-De Xue and Sandra A. Allan

Attractive toxic sugar bait (ATSB) and *non*-attractive toxic sugar baits (on bait stations and sprayed on vegetation) were compared in their efficacy to control *Aedes albopictus* populations in St. Augustine in 4 different tire dumps. Attractive baits controlled populations significantly while *non*-attractive bait had little to no impact.

220 Update on nectar and sugar feeding in mosquitoes and stable flies in Florida
Dan Kline and Jerry Hogsette

The extent of nectar and sugar feeding has long been overlooked in haematophagous insects and the importance of this phenomenon has been reflected in the results from Florida in mosquitoes and stable flies. Attraction of mosquitoes to a number of flowers has been shown in the laboratory and in the field and the distribution of mosquitoes may be altered by the flowers, especially in urban settings where flowering plants were widely used in the landscape. Stable flies on the other hand have been shown to use flowers on native shrubs. The tendency for stable flies to visit flowers on landscape plants has been difficult for us to document because the peak stable fly season in much of Florida occurs during the winter months when few landscape plants are in bloom. Some current findings and plans for future work will be discussed in the presentation.

221 *Anopheles gambiae* performance in mesocoms: effects of plant-community composition
Bryan T. Jackson, Christopher M. Stone and Woodbridge A. Foster

Experimental cohorts of *Anopheles gambiae* in mesocosms are providing indications of how plant communities can affect biological factors critical to malaria vectorial capacity. So far, it appears that vectorial capacity is balanced on a knife-edge and can be tilted in either direction by opposing tendencies of nectar-producing plants to prolong survival but also to decrease biting frequency.

222 Sugar feeding and water drinking: oral administration and application of toxic baits in Florida
Riu-De Xue

Sugar feeding and water drinking in adult mosquitoes are a major part of daily activity. In the laboratory, adult *Aedes albopictus* will survive longer on a diet of water compared to a diet of dry sugar alone. The addition of a water source with a dry sugar source increases mosquito survival. This suggests that sugar in a liquid state supports mosquito survival. Based on sugar feeding and water drinking behaviors, the administration of an oral toxin/insecticide has potential in mosquito control programs. Attractive toxic sugar baits (ATSB) have been developed and applied in different areas of St. Johns County, Florida. The past, present, and future prospects of using the ATSB in vector control programs will be discussed.

223 Attractive toxic sugar baits (ATSB) and non-target organisms
Günter C. Müller, Edita E. Revay, Vasiliy D. Kravchenko, Axel Hausmann, Zoya Efremova, John Beier and Yosef Schlein

The results of ATSB trials from Florida, Israel and Morocco are summarized. Extensive data regarding potential impact of the new control method on non-target arthropods are discussed in detail. In short if ATSB is applied in bait stations or sprayed on non flowering green vegetation the impact on non-targets is low while spraying on flowering vegetation is in particular harmful for pollinators.

224 Application of attractive toxic sugar baits (ATSB) on bait stations in Mali for mosquito and sand fly control
Mohamed M. Traore, John C. Beier, Sekou F. Traore, Seydou Doumbia, Yosef Schlein and Günter C. Müller

The work of several years is summarized and new results of ongoing experiments will be presented for the first time.

225 Large scale control of *Anopheles crucians* with attractive toxic sugar bait (ATSB) barrier treatment and impact on non-target organisms in St. Augustine Florida

Whitney Qualls, Gunter C. Muller, Edita E. Revay, Vasiliy Kravchenko, Zoya Efremova, Axel Hausmann, Mike Smith, John Beier and Rui-De Xue

A large scale application of attractive toxic sugar bait (ATSB) was made to a golf course community in St. Augustine, Florida. Mosquito populations were monitored before and after application using CDC traps baited with dry ice. The most abundant mosquito species collected was *Anopheles crucians* at both the control and treatment sites. *Anopheles crucians* populations were reduced significantly at the treatment site compared to the control site. This reduction was monitored for up to four weeks post-application. Non-target experiments were also conducted during this evaluation and the ATSB application had no significant impacts on non-target populations.

Eastern Equine Encephalitis Virus: Current Research and Trends I

226 A brief history of eastern equine encephalitis virus in New Jersey and its role in the formation of New Jersey's vector surveillance program

Heather A. Lomberk, heatherlo@co.cumberland.nj.us

In New Jersey, there have been several outbreaks of eastern equine encephalitis virus (EEEV), the first happening in 1959 with 32 confirmed human cases, 22 of these resulting in death. Subsequently, there were 3 more in 1965, 1967, and 1968. Humans, horses, and game birds were all affected, and panic and fear led to huge economic losses. In response, mosquito control professionals conducted massive air sprays, but it became necessary to search for a more effective solution. In 1975, an interagency vector surveillance program was developed to monitor EEEV and its mosquito vectors at a number of study sites. Funded by the New Jersey State Mosquito Control Commission, this program helped pinpoint areas of activity to determine control strategies and risk of transmission to humans. The arrival of West Nile virus and other associated encephalitic viruses led to the expansion of this program, which endures even today. As specimens are tested for these viruses, data is collected, transmitted via a web-based program, and is reported to all agencies involved. Since its inception, confirmed human cases have drastically declined, and area-wide emergency air sprays have not been necessary. This vector surveillance program has emerged as one of New Jersey's most valuable components of its mosquito control operations, and it continues to be a crucial tool in the protection of its residents.

227 **Vector-host interactions and resurgence of eastern equine encephalitis virus in northeastern USA**

Goudarz Molaei, goudarz.molaei@ct.gov

Eastern equine encephalitis virus (EEEV, family Togaviridae, genus *Alphavirus*) is a highly pathogenic mosquito-borne agent responsible for periodic outbreaks of severe disease in humans and equines, and causing high mortality and severe neurologic impairment in most survivors. During the last decade, episodes of EEEV have reemerged in the northeastern US including Massachusetts, New York and very recently Vermont with increased virus activity and human and equine cases. EEEV is amplified in an enzootic cycle involving ornithophilic mosquitoes, principally *Culiseta melanura* (Coquillett), and Passeriformes birds in freshwater swamp habitats. Although EEEV frequently infects *Cs*. *melanura*, this species is considered an unlikely bridge vector because it feeds mainly on birds. However, our recent analyses of *Cs. melanura* sampled from EEEV foci in northeastern US revealed that 1-10% of blood meals were obtained from mammals including equines and humans, suggesting the ability of this species to serve as a bridge vector. Other mosquito species are often considered as bridge vectors, but the supporting evidence is circumstantial. Vector-host interactions of enzootic and epidemic/epizootic vectors of EEEV, critical factors that potentiate annual reemergence, amplification and risk of human infection, and the contribution of various bird species to amplification of the virus will be discussed.

228 Shifts in dynamics of eastern equine encephalitis virus activity in central New York

Laura D. Kramer, kramer@wadsworth.org, Susan A. Jones, Alan P. Dupuis, Joseph G. Maffei, Steven D. Zink, JoAnne L. Oliver and Bryon Backenson

Eastern equine encephalitis virus (EEEV;Togaviridae: *Alphavirus*) is a highly pathogenic mosquito-borne virus that produces severe or fatal encephalitis in infected humans and horses. Recently, EEEV has undergone a regime shift in dynamics in the northeastern US. For 3 decades (1970-2000), periodic activity was detected in central NY, specifically in counties surrounding Oneida Lake, with 1-2 years of activity in mosquitoes and other hosts, followed by 1-6 years with no detectable activity. However, since 2003, there have been 10 consecutive years with active EEEV transmission, notwithstanding very diminished activity in NY in 2012. The string of 10 consecutive years is a highly unlikely event to occur by chance. The current epizootic coincides with the invasion of WNV to the region, and occurs within the backdrop of long-term trends in temperature and declines in key songbird populations. The first human case in NY state in 26 years occurred in 2009, then again in 2010 and 2011. From 2008-2011, *Culiseta melanura* MIR was ~4.1. However, in 2012, only a single *Cs. melanura* pool was found infected. Phylogenetic analysis of isolates indicates the virus is periodically introduced, most likely by migratory birds, but also overwinters in the central NY focus. The virus also appears to be extending its range and intensity outside the historic focus in NY. It remains to be seen whether the almost complete lack of activity in 2012 represents the end of this cycle of activity in NY state.

229 Tracking eastern equine encephalitis virus evolution, overwintering, and spread in North America by phylogenetic analysis

Philip M. Armstrong, philip.armstrong@ct.gov

Eastern equine encephalitis virus (EEEV) causes periodic outbreaks of severe disease in horses and humans in eastern North America. The mechanisms responsible for the seasonal reemergence of EEEV in endemic sites are not well understood but could derive from locally overwintering virus or from annual re-introduction of new virus strains. By phylogenetic analysis of EEEV sequences, we may track the spread, local persistence, and evolution of viral variants to better understand the origin of disease outbreaks. Prior studies show that North American strains of EEEV are genetically distinct from South American strains and likely represent separate species. North American viruses sampled over a 70-year period were found to exhibit limited spatial structure by phylogenetic analyses and tend to group by year of isolation with newer strains differing most from ancestral strains. Recent studies have focused on the patterns of viral clade replacement over time in northern and southern locations. Collectively, they indicate that EEEV variants may overwinter in the northeastern US but eventually go locally extinct after a period of 1-5 years. Northeastern populations of EEEV share recent common ancestry with strains circulating in the southeastern US, suggesting long-range viral dispersal among these locations is required for new virus introductions. These findings may help explain the periodicity of EEEV outbreaks in northern regions that tend to occur over 2-5 year time intervals.

Larval Control II

230 Controlling malaria mosquitoes with a long-lasting *Bacillus sphaericus* (*Bsph*) and *Bacillus thuringiensis* var *israelensis* (*Bti*) formulation

Yaw Afrane, yaw_afrane@yahoo.com, Andrew Githeko and Guiyun Yan

Changing vector behavior requires all control strategies be used against malaria. Larval control is one of the oldest and most successful. However, available larvicides have a short control duration, and high re-treatment expense. A biological long-lasting larvicide is highly desired. A FourStar™ 180-day *Bacillus sphaericus* (*Bsph*) and *Bacillus thuringiensis israelensis* (*Bti*) briquet was evaluated under semi-natural and natural conditions in western Kenya. In semi-natural habitats, 2nd-instar larvae of *Anopheles gambiae* were introduced with briquet particles in rainwater at appropriate concentrations and number of pupae were recorded daily. Treatments to productive field habitats were made before the malaria season to 3 study areas, 3 others were used as controls. We found 100% mortality within 48 h of 2nd instar larval introductions in semi-natural habitats. The *Bsph/Bti* formulation killed larval mosquitoes for 6 months. Briquets killed larvae for 5 months in natural habitats, despite the effects of rain. Briquet treatments reduced the density of mosquitoes in houses from between 60-80% in the intervention areas during the transmission season. Larval control has the potential to reduce the population of malaria mosquitoes. The FourStar™ *Bsph/Bti* briquets present a promising biological formulation to use for larval control. This formulation is highly recommended to the National Malaria Control programs.

231 Evaluation of an area-wide, truck mounted application of FFAST *Bti* in Monmouth County, New Jersey

Taryn N. Crepeau, tcrepeau@co.monmouth.nj.us, Sean P. Healy, Ary Farajollahi, Gregory Williams, Randy Gaugler and Dina Fonseca

Aedes albopictus was first discovered in New Jersey in 1995 in the Raritan Bayshore municipality of Keyport in Monmouth County. Since that time, it has proliferated throughout much of Monmouth County and New Jersey. Individual larval habitats range in size from a plastic cup to a water filled boat cabin and are abundant throughout most neighborhoods. To efficiently address these habitats, truck mounted applications off FFAST™ *Bti* were made in August of 2012, to a suburban neighborhood in Middletown Township. The applications were made with the Adapco Guardian 190 ES cold aerosol generator. Spray cloud movement and product efficacy were evaluated using bioassay cups positioned around houses within the spray zone. Product formulation, application methodologies, and efficacy assessments will be discussed.

232 The anatomical characteristics of mosquito larvae lending susceptibility to Acoustic Larvicide©

Herbert Nyberg, sales@newmountain.com, Jiang-Shiou Hwang and Cheng-Han Wu

Acoustic Larvicide© was discovered and patented by New Mountain Innovations. Larvicidal activity occurs when sound energy of sufficient power is introduced into the water habitat of any species of mosquito larvae at the resonant frequency of its internal air volume. This paper will discuss the unique anatomical characteristics of the larvae that make them susceptible to this energy which causes death. Of major importance was the discovery, as a result exposure to acoustic energy, of the overpressure of the dorsal tracheal trunk and the importance of this characteristic throughout the anatomical life of the larvae. High-speed macro video recording was employed to document the primary effects of Acoustic Larvicide© exposure. This paper will provide proof that this existing high-pressure air is the driving mechanism for emergence.

233 Employment of Acoustic Larvicide© for mosquito larval control in Taiwan

Herbert Nyberg, sales@newmountain.com, Jiang-Shiou Hwang, Kadarkarai Murugan, Li-Chun Tseng and Kandasamy Kalimuthu

In October 2012 Acoustic Larvicide© was applied in various habitats through the country of Taiwan from the most southern point in Taiwan, through the central region to the most northern. The field study included repeated

samplings for statistical comparison. Larvae treated from habitats with Acoustic Larvicide© were collected for laboratory analysis. The results of both field tests and laboratory experiments indicate that the Acoustic Larvicide© can be an effective tool for controlling mosquito populations in Taiwan's urban and rural habitats. This paper will present the results of first application of Acoustic Larvicide© conducted country wide in Taiwan.

234 CocoBear™ - new larvicidal oil proves to be superior successor to Golden Bear
Ben Goudie, Mike Willis and Marie Saunders

Clarke introduced CocoBear in 2012. This paper will review the product's development path and goal, along with field trial and operational data.

235 Application methods and efficacy of CocoBear™ mosquito larvicide oil
Jacob W. Hartle, jhartle@clarke.com, Grifith S. Lizarraga and Derek Drews

The CocoBear™ Mosquito Larvicide Oil formulation is single brood mosquito larvicide oil that is designed to treat against all larval instars and pupae with a physical control of mosquito broods. This presentation will discuss 3 key components to maximize the application for ultimate efficacy: application methods, application timing, and compatible habitats for optimal effectiveness.

236 The evolution of GPS mediated aerial mosquito larviciding: Lee County Mosquito Control District
Jonathan Hornby, hornby@lcmcd.org, Gene Sutton and Wayne Gale

This paper discusses the development of a "Show-me where to spray and I'll spray it" aerial larviciding program to a "Draw a polygon - Follow the light bar - Auto Spray ON/OFF" program with a complete spray track record. Several years of transition among field inspectors, pilots and AG-NAV software developers have culminated in a precise, efficient and contemporary approach to aerial mosquito larviciding.

237 Helicopter auto-loader: creation of a large-scale portable machine to automate loading of granular larvicides
Michael S. Doyle, mdoyle@keysmosquito.org, Richard Heiser and Kurt Joseph

The Florida Keys Mosquito Control District (FKMCD) has developed a helicopter loader to more efficiently load their Bell 206-B and 206-L4 helicopters. The "auto-loader" consists of a modified agricultural grain hopper with an extendable auger shoot, mounted on a 5th-wheel trailer. It is fully highway worthy and weatherproof. It requires 1 person to operate in the field compared to the previous method of 6 to 8 people manually loading 40 lb bags. This system is currently being used to load VectoBac GS *Bti* granules but will accommodate a variety of mosquito control larvicides. The VectoBac GS granules arrive in 1200 lb bulk bags and the auto-loader will hold >7,200 lb (i.e., 6 bulk bags). In addition, the trailer has a 300gal Jet A fuel tank, hopper-mounted scale for accurate loading of aircraft by weight, and additional space to accommodate 2 pallets of 40 lb bagged VectoBac GS granules. The auto-loader has been very successful in reducing hours of manual bag loading by field inspectors while simultaneously increasing their ability to search for and more accurately delineate larval sites. The FKMCD is in the process of constructing a second auto-loader.

238 Development of an automated compression sprayer test stand
Wesley C. Hoffmann, clint.hoffmann@ars.usda.gov, Muhammad Farooq and Brad Fritz

Hand-held or backpack compression sprayer are routinely used to spray agricultural and vector control sprays. Because these sprayers are manually pressurized, it is important that these sprayers hold pressure to reduce the workload on the human operator and do not leak to prevent accidental exposure of the operator to the chemical being sprayed. An automated compression sprayer test stand was designed and constructed to evaluate how well compression sprayers hold up to repeated pressurizations of the spray tank and how well they hold the pressure over time. The maximum pressure, hold time and number of cycles can be adjusted in the system through control software. Each of the 6 commercially-available sprayers was filled to standard operating pressure, the pressure was held and monitored for 5 min, and then the sprayer was decompressed. This process was automatically repeated 50 times with each sprayer. These results will help user select sprayer that will hold up to the rigors of spraying under field conditions. The test stand will also help find vulnerabilities of the sprayer system, if any.

Education/Outreach

239 The biggest room in the world...
Nicole Williams, nicole@killmosquito.org

Today in a world of advancing science and technology there seems to be a problem that hinders our main objective, which is advancing the fight against mosquito disease. This is a review of the problems that occur in operational research, obtaining funding, NGOs and governments. We will share what AirWolf has come up with as the solution that will help advance the progress against one of the world’s deadliest insect…the mosquito.

240 The rage of the dog star
Neil Wilkinson, nwilkins@fgcu.edu

This presentation on malaria is a condensed version of a lecture used in public education classes and community events. It investigates theories of malaria's evolutionary origin, past and present impacts on societies, and initiatives

to reduce its global disease burden. It was created to raise awareness of the disease providing a simplistic biological overview so people can understand its historic and current relevance.

241 Jersey roots, global reach: outreach in mosquito research and control offered by the Center for Vector Biology
Scott C. Crans, scrans@aesop.rutgers.edu

The New Jersey Agricultural Experiment Station has been caught up in stakeholder training since the beginning of the mosquito control movement. Over time, the training programs delivered have evolved with the changing times. This paper covers routine outreach activities and recent initiatives with an assessment of their impact shaping professional mosquito control in New Jersey.

242 100 years of mosquito control in Connecticut
Paul Capotosto, pcapotosto@snet.net

The State of Connecticut's Mosquito Control program has existed for 100 years. Its original purpose was to prevent outbreaks of malaria. It has developed into its present involvement in the prevention of West Nile fever and eastern equine encephalitis. During most of the past century, the program was part of the State Department of Health. Although the program is currently a unit of the Connecticut Department of Energy and Environmental Protection, it remains a large component of the state's commitment to the prevention of human illness. The mosquito control program has changed as the state and nation have throughout the 1900s, and its history reflects the societal, political and economic periods we have endured.

243 Florida State University is now in the mosquito control research, training, and technical service business
John P. Smith, jsmith@pc.fsu.edu

Several services once offered at the Public Health Entomology Research and Education Center (PHEREC) of Florida A&M University have now been transferred to the Panama City campus of Florida State University. These services include: training mosquito/arbovirus surveillance and testing larvicides, adulticides, repellents, and trap systems. Custom resistance evaluation services can also be arranged. More information on obtaining these services and keeping up with FSU entomology programs can be accessed at new web pages located at http://pherec.org. This site will soon change to http://insecthelp.com. Additional news and developments concerning the old PHEREC site and FSU programs will be presented.

244 FMCA's Tallahassee Day overview and current legislative issues
Dennis Moore, dmoore@pascomosquito.org and Douglas B. Carlson

A review of FMCA's legislative activities from the past year will be reviewed. The legislative plan moving forward will be discussed with the hope of restoring money for research and support for Florida mosquito control programs.

245 A history of AMCA's education day
Eric D. Jackson, ericdj@leeschools.net, Brian Murphy and Neil Wilkinson

Each year at the national meeting of the AMCA, volunteers converge at a local elementary school in the host city and visit every class. AMCA members from around the country and beyond teach lessons on the mosquito life cycle, disease, and control methods. Children participate in lectures, discussions, and hands-on activities. Local districts provide support by bringing equipment, vehicles, traps, and specimens. This event supports a long tradition of AMCA's commitment to education as part of comprehensive mosquito control.

245 *Wing Beats:* Q&A
Stephen L. Sickerman, sickerman@comcast.net and Jack Petersen

All AMCA members are encouraged to submit articles to *Wing Beats* magazine, the official trade journal of the American Mosquito Control Association and the Florida Mosquito Control Association. Each quarterly issue typically runs 40 to 48 pages, with a national and international circulation of almost 4,000. For the benefit of prospective authors, editorial and publishing guidelines, requirements and schedules regarding manuscripts, photographs and other graphic images will be discussed.

247 Using social media during the Dallas WNV outbreak and aerial spraying response
Caleb T. Stitely, cstitely@dynamicaviation.com

A discussion on Twitter's role in public education and communication during the aerial response to WNV in Dallas and Denton counties including its power within media outlets

Eastern Equine Encephalitis Virus: Current Research and Trends II

248 Using deer and moose sero-surveys to detect EEEV activity
John-Paul Mutebi, Grv0@cdc.gov

Vertebrate hosts are frequently used for eastern equine encephalitis virus (EEEV) surveillance, but the commonly used vertebrates are bird populations associated with fresh-water swamp forest habitats. Several recent studies have reported evidence of EEEV infections in wild white-tailed deer (*Odocoileus virginianus*). Since white tailed-deer are ubiquitous in North America, have limited home ranges and are harvested annually we explored the possibility of

using them to detect and study EEEV activity. In the past 3 years, we have screened more than 2,500 white-tailed deer sera collected in Maine and Vermont for EEEV antibodies by using plaque reduction neutralization tests (PRNT). We have detected EEEV antibodies in 7 - 16% of the serum screened suggesting substantial circulation of EEEV in these 2 states. In Maine, EEEV antibody positive sera was detected as far north as Fort Kent at the border with Canada suggesting a much broader EEEV activity distribution in the state than originally thought. In Vermont, the first detection of EEEV activity in the state was through screening deer and moose sera collected in 2010, and 12 of the 14 counties (85%) tested positive suggesting statewide EEEV activity in Vermont. Analysis of the spatial distribution of PRNT positive samples revealed a random distribution of EEEV throughout Vermont, but a patchy and discontinuous distribution in Maine.

249 New insights into over-wintering and early season amplification of EEEV

Hassan K. Hassan, hhassan@health.usf.edu, Nathan Burkett-Cadena, Andrea Bingham, Gregory White, Sean Graham, Craig Guyer, Eddie W. Cupp and Thomas R. Unnasch

In the northeastern USA, EEEV appears to be maintained through periodic introductions and when introduced to a focus, continues to circulate for several years. The mechanism through which the virus over-winters has remained obscure. In studies conducted in a EEEV focus in the Tuskegee National Forest (TNF) in Alabama, EEEV was detected in early season mosquito species thought to feed primarily upon ectothermic vertebrates, including snakes, reptiles and frogs. Identification of blood meals taken by these species revealed that they fed primarily upon abundant ectotherms present at the site, including frogs, anoles and snakes. The most frequently targeted ectotherm was the cottonmouth moccasin, a snake that was abundant at the site. Laboratory reservoir competency studies demonstrated that snakes were competent hosts, maintaining infectious viral titers in their blood for prolonged periods. Furthermore, infected animals induced to enter and exit hibernation maintained a circulating virema. Field investigations at TNF revealed that roughly 35% of the cottonmouths produced antibodies against EEEV and 15% contained RT-PCR detectable virus in their circulation. These results suggest that snakes represent competent hosts for EEEV and suggest a role for snakes in over-wintering of the virus.

Poster Abstracts

Adult Control

P-01 The biological effect of cage design corrected for reductions in spray penetration

Bradley K. Fritz, brad.fritz@ars.usda.gov, Wesley C. Hoffmann and Jane A. Bonds

In-the-field measures of physical concentration of applied sprays do not typically correlate well with observed caged mortality data. It is postulated that cage filtration of the spray may be one potential cause of this inconsistency. Wind tunnel studies were conducted to investigate the accuracy of calculations developed to correct for filtration levels in cage mosquito bioassays. Etofenprox was applied at rates ranging from an LD_{10} to an LD_{90} to 3 cage types with significantly different filtration levels. The dose approaching the cage was converted to a dose entering the cage using cage penetration data from previous research. The filtration conversion factor returned a data set that directly correlated dose with mosquito mortality (R^2 0.918). The percent mortality was a function of dose within the cage, which was regulated by mesh type. Although the conversion factor was effective, the differences between cages was not always significant because of the within-group variation. Results demonstrate the impact that changes in spray exposure levels versus biological response were not as clear-cut as the physical characterization. The question that arises is whether cage-to-cage variations mute potential differences between different cage types. Regardless, correcting field measured data allows not only better comparisons between differing studies, but also results in better estimates of insect mortality versus actual spray concentration exposure levels.

P-02 X-ray induced sterility in *Aedes albopictus* (Diptera: Culicidae) and male longevity following irradiation

Hanano Yamada, h.yamada@iaea.org, Marc Vreysen, Andrew Parker, Clelia Oliva, Fabrizio Balestrino and Jeremie Gilles

The mosquito *Aedes albopictus* is a potent vector of several arboviral diseases, most notably chikungunya and dengue fever. In the context of the sterile insect technique (SIT), the sterilization of the male mosquitoes before their release can be achieved by gamma-ray irradiation. As gamma-ray irradiators are becoming increasingly problematic to purchase and transport, the suitability of an X-ray irradiator as an alternative for the sterilization of *Ae. albopictus* males was studied. The sterilization of up to 200,000 pupae can be achieved with relative ease, and the sterility results obtained were comparable to those achieved by gamma irradiation, where 99% sterility is induced with a dose of 30-35 Gy. A significant reduction of male longevity towards the end of the males' life was observed following irradiation treatments at doses higher than 40 Gy, consistent with the negative effects on longevity induced by similar radiation doses using gamma rays. Overall, it was found that the X-ray irradiator is generally suitable for the sterilization process for SIT programs, as it showed a high processing capacity, practicality, high effectiveness and reproducibility.

P-03 Spray equipment selection guide for ground adulticide applications
Muhammad Farooq, muhammad.farooq@med.navy.mil

Adulticide labels describe the limitations of applications within a range of conditions. Fog applications sometimes are even limited to 1 flow rate, dilution rate, travel speed, and swath width. Spray application equipment is generally designed to work within a range of pressures, flow rates and travel speeds. Testing of many sprayers and foggers for droplet size characteristics over the last few years revealed that full capabilities for the majority of the equipment cannot be utilized because of one set standard or limitation based on the pesticide label. The selection guide is a set of Microsoft Excel spreadsheets listing the range of capabilities of spray equipment, from among the tested pieces, suitable for an adulticide. One sheet is dedicated to each adulticide and the range of capabilities of vehicle mounted, backpack and hand-held sprayers/foggers are selected to match the pesticide label limitations. The listed parameters include pressures, flow rates, travel speeds and dilution rates. The guide after completion will be available at DOD Equipment Help Desk (http://www.afpmb.org/content/dod-equipment-helpdesk) and will be incorporated into the smart phone app "Vector Sprays", available free for iphone and Android systems.

P-04 Evaluation of barrier treatment persistence on different vegetation found in a southern California desert habitat
Gregory S. White, gwhite@cvmvcd.org and Melissa Snelling

Studies conducted in the desert habitat of Coachella Valley, CA demonstrated that synthetic pyrethroids are effective as barrier treatments on local vegetation in controlling mosquitoes. In many mosquito abundant areas in the District, the local vegetation is mostly a monoculture. The dominant plant species at a given location varies from place to place, but only 3 to 4 plant varieties form the majority of natural barriers. The District wanted to determine if there were significant differences in how long adulticides remained effective on common plants in our habitat. This knowledge could help operational activities by improving barrier treatment location selection and determining good reapplication intervals. We identified 3 of the most abundant plants near mosquito habitats - *Atriplex canescens* (Pursh), *Pluchea sericea* (arrowweed) and *Tamarix chinensis* (tamarisk). Lambda-cyhalothrin was applied to vegetation using the District's standard mist blower. Clippings of the vegetation were taken prior to the spray, 1 h and 72 h post-spray, then at 7 day intervals until 42 days post-spray. These vegetation clippings were used in bioassays with colony reared mosquitoes. CO_2 traps were used to monitor mosquito abundance at the spray sites at the times of vegetation sampling. The initial results of the bioassay performed in this study indicated that the barrier treatment was most effective and longest on *T. chinensis* and was least persistent on *A. canescens*.

Behavior/Biology

P-05 Development of octenol and CO_2 detection in female *Aedes aegypti* mosquitoes
Jonathan D. Bohbot, jonathan.bohbot@ars.usda.gov and Joseph C. Dickens

During the first 24 h of their adult life, female *Aedes aegypti* mosquitoes do not respond to host odors, neither do they blood-feed nor mate. This developmental delay is manifested in multiple traits including the olfactory system. We have investigated molecular, physiological and behavioral aspects associated with octenol detection and attraction in newly emerged, 6- and 10-day old female *Ae. aegypti* mosquitoes. Our results indicate that the development of adult mosquitoes extends beyond the pupal stage into early adult life. Our study further suggests that behavioral responses to octenol and CO_2 are regulated by both maturation of olfactory neurons and modulation of effector pathways within the central nervous system.

P-06 New Jersey statewide adult mosquito surveillance program, 2012
Lisa Reed, lreed@rci.rutgers.edu, Scott Crans and Mark Robson

Using county mosquito control agency data, Rutgers has provided the State of New Jersey public information on mosquito population patterns for several pestiferous and public health mosquito species. In 2012, populations of floodwater species were lower than normal in several regions. *Aedes vexans*, normally one of the most abundant species in New Jersey, was in the top ten for only 1 region. *Culex* populations were also depressed in several regions, but the response was not consistent throughout the state or through time. *Culiseta melanura* patterns in light traps did not necessarily match patterns observed in resting boxes. Population patterns for enzootic vectors of arboviruses are presented in conjunction with positive disease pools.

P-07 Taste of repellents in mosquitoes
Joseph C. Dickens, joseph.dickens@ars.usda.gov, Jillian L. Sanford and Vonnie D. Shields

Repellents are widely used to limit interactions between arthropod disease vectors and their animal hosts. The most widely used repellent, DEET, is thought to exert its effects by modulating the responses of olfactory receptor neurons (ORNs) in mosquitoes, thereby scrambling messages being sent to the brain resulting in behavioral disruption. DEET and other repellents may directly stimulate responses in ORNs in mosquitoes or modify the responses of ORNs to attractants. More recently, DEET was shown to elicit responses from gustatory receptor neurons (GRNs) sensitive to feeding deterrents in *Drosophila*. We now show that DEET and other repellents elicit responses from GRNs in the yellow fever mosquito, *Aedes aegypti*, providing a neural correlate for feeding

deterrence observed for these compounds. The involvement of multiple sensory channels in the detection of repellents correlates well with behavioral responses of mosquitoes to these compounds and provides a basis for behavioral studies aimed at the discovery of novel compounds with repellent activity or enhancement of known repellents.

P-08 What's causing the population explosion of *Culex erraticus* and *Anopheles quadrimaculatus* in Minnesota?
Sandy Brogren, sandybrogren@mmcd.org, Diann Crane and Carey LaMere

Until the past 10 years, *Culex erraticus* and *Anopheles quadrimaculatus* occurred at very low levels in the Metropolitan Mosquito Control District (MMCD). The only record of *Cx. erraticus* larvae in the MMCD was a larval sample from 1961. Adult specimens went undetected until 1988 and occurred sporadically thereafter. *Anopheles quadrimaculatus* occurred in the first 10 years of the District, and were only captured in 3 out of the next 30 years. Within the past 10 years, we have seen an increase in the frequency of occurrence and abundance of both species. In fact, sharp increases were detected in 2012. For the first time in 51 years, larval *Cx. erraticus* were discovered in multiple locations and adult populations soared to record levels in trap collections throughout the MMCD. *Anopheles quadrimaculatus* also occurred in record-breaking numbers in 2012. The cause of these species' increased abundance and frequency of occurrence may be the result of weather patterns that allowed them to expand their ranges and increase their productivity. We will discuss the type of larval habitats associated with these species and the implications of these range expansions.

P-09 Vertical distribution of container-inhabiting *Aedes* species in a La Crosse virus endemic area
Michael Riles, mtriles1@catamount.wcu.edu, Hope Mason, Bruce Harrison and Brian Byrd

The eastern treehole mosquito (*Aedes triseriatus*) is the principle vector of La Crosse virus (LAC) in western North Carolina where the sibling species (*Ae. hendersoni*) is found sympatrically. Two invasive and LAC competent mosquito species (*Ae. albopictus* and *Ae. japonicus*) are also well established in the region. During 2011 and 2012, a series of vertical distribution studies were conducted using oviposition traps ranging in height from 0 to 9 m. Our analyses suggest that: 1) the oviposition activity of *Ae. triseriatus* and *Ae. hendersoni* is not strictly segregated by height; 2) the invasive species (*Ae. albopictus* and *Ae .japonicus*) oviposit more frequently at or near the ground level; and 3) in some instances, accurate identification of *Ae. triseriatus* and *Ae. hendersoni* larvae is limited by hybrid morphological characters. These data are primarily discussed in the context of surveillance efforts that use oviposition traps in LAC endemic areas. Ongoing molecular studies addressing the identification of these species and their hybrids will also be presented.

Disease/Vector Studies

P-10 Comparison of West Nile virus assays in Illinois: what is Error 2?
Nina M. Krasavin, krasavin@illinois.edu, Richard Lampman and Ephantus J. Muturi

From June until September 2012, mosquito abatement districts (MADs) in northern Illinois faced an unprecedented amplification of WNV-positive mosquito pools. For 2 MADs a frustrating problem arose regarding the testing of samples; a small proportion started to produce Error 2 messages on their RAMP assay. Systematically they attempted to eliminate the possibilities and eventually the only one left was "Analyte levels significantly higher than the upper reportable range." By the end of 2012, there were over 90 samples that yielded this "Error 2" message and in some weeks it accounted for almost one-quarter of the samples. An assay of the samples by RT-PCR at the Illinois Natural History Survey found all except 1 were positive. Several MADs started to report they could dilute the samples producing an error message and they always produced a positive RAMP. A comparison of mean cycle threshold for the error message samples and samples listed as >640 RAMP was significant with a mean of 24.7 for the Error 2 samples and 26.1 for the >640 samples. It appears that the infection rate reached such a magnitude that multiple WNV-infected mosquitoes were in some samples which exceeded the limits of the RAMP assay.

P-11 Detection of *Babesia microti* and *Borrelia burgdorferi* and in host-seeking *Ixodes scapularis* in Monmouth County, New Jersey
Vivien E. Roegner, roegner@aesop.rutgers.edu, Sean P. Healy, Terry L. Schulze and Robert A. Jordan

The etiological agents that cause human babesiosis (*Babesia microti*) and Lyme disease (*Borrelia burgdorferi*) share a common tick vector (*Ixodes scapularis*) and rodent reservoir (*Peromyscus leucopus*), but because the geographical distribution of babesiosis is more restricted than Lyme disease, it was not considered a nationally notifiable disease until 2011. Although recent studies have shown dramatic increases in the number of cases of babesiosis and expansion of its range, little is known about infection and co-infection prevalence of these pathogens in the primary tick vector. The infection prevalence for *B. microti* and *B. burgdorferi* in *I. scapularis* nymphs and adults collected within 6 Monmouth County, NJ municipalities was investigated. The potential public health importance of these findings is discussed.

P-12 Evaluation of a RAMP/RT-PCR West Nile virus (WNV) detection system in mosquito pools
Kristen L. Burkhalter, ktb3@cdc.gov, Harry M. Savage and Roger S. Nasci

We evaluated the sensitivity and efficiency of a commercially available WNV antigen detection assay (RAMP WNV test, Response Biomedical Corp., Burnaby, British Columbia, Canada) and real-time RT-PCR using a proficiency

panel consisting of WNV-spiked mosquito pools and negative control pools. The panels were sent to 20 mosquito abatement districts (MADs) that processed the pools in RAMP buffer and tested them with the RAMP assay, then sent the panel to molecular laboratories for confirmation by RT-PCR. In addition, we conducted a series of experiments on laboratory-prepared WNV positive mosquito pools of various titers to compare mosquito homogenization buffers, pool sizes, and grinding methods to determine how these variables affect the RAMP and RT-PCR assays. Using the data collected from the MADs and labs and the results from our in-house processing experiments, we were able to determine sensitivity limits of a RAMP/RT-PCR WNV detection system in mosquitoes, and we developed recommendations for best practices and appropriate RAMP cut-off values that could be implemented by MADs.

P-13 La Crosse virus infection alters blood feeding behavior in *Aedes triseriatus* and *Aedes albopictus* (Diptera: Culicidae)

Bryan T. Jackson, brjacks3@gmail.com, Carlyle C. Brewster and Sally L. Paulson

The effects of La Crosse virus (LACV) infection on blood feeding behavior in *Aedes triseriatus* (Say) and *Ae. albopictus* (Skuse) were investigated in the laboratory by measuring the size of the bloodmeal imbibed and the extent of refeeding by virus-infected and uninfected mosquitoes. LACV-infected *Ae. triseriatus* and *Ae. albopictus* took significantly less blood compared with uninfected mosquitoes. Twice as many virus-infected *Ae. triseriatus* mosquitoes refed compared with uninfected individuals (18% vs 9%, $P<0.05$); however, virus infection had no significant effect on the refeeding rate of *Ae. albopictus*. Reduction in blood meal size followed by an increased avidity for refeeding may lead to enhanced horizontal transmission of the LACV by its principal vector, *Ae. triseriatus*.

P-14 Phlebotomine sand flies (Diptera: Psychodidae) from the Biosphere Montes Azules in the Lacandonia tropical forest, Chiapas, México

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The natural reserve of the biosphere known as Montes Azules is located in the core of the Lacandonia tropical forest in the state of Chiapas, México. This bioreserve comprises one of the most important areas of the tropical forest in Mesoamerica and it is known that this reserve possesses a high biodiversity in animal and plant species. As part of a diversity inventory in the field station of Chajúl, we collected specimens of phlebotomine sand flies. Catches were conducted for 3 consecutive days in June, 2012 using light CDC traps. Sand fly processing and identification was carried out using standard curatorial protocols. All specimens are housed in the Entomological Collection of the Laboratory of Medical Entomology at the Autonomous University of Nuevo Leon. A total of 153 specimens were caught and these were identified as *Lutzomyia aclydifera*, *Lu. carpenteri*, *Lu. cruciata*, *Lu. olmeca olmeca*, *Lu. panamensis*, *Lu. shannoni*, and *Lu. vindicator*. In CDC traps, the most commonly collected species was *Lu. aclydifera* (82%). It is important to highlight the fact that 4 species *Lu. cruciata*, *Lu. olmeca olmeca*, *Lu. panamensis* and *Lu. shannoni*, are incriminated as vectors of *Leishmania mexicana* in other regions of southeastern México. At this stage, it is unknown whether these species may maintain enzootic transmission cycles in the Lacandonia tropical forest.

P-15 Vector surveillance of arboviruses in New Jersey, 2012

Lisa Reed, lreed@rci.rutgers.edu, Scott Crans and Mark Robson

New Jersey has employed a vector surveillance program since 1975. In 2012, both West Nile virus (WNV) and eastern equine encephalitis virus (EEE) experienced increased activity. West Nile virus surveillance began earlier than normal because of signs of early activity in the year. At the time of this writing, 980 positive pools of WNV were detected, far greater than any previous year. At least 38 human cases occurred, along with multiple horse cases. Factors that precipitated such activity may include unusual weather patterns experienced the previous winter and spring. Efficacy of bird infections is considered. EEE activity was also higher, with positive pools including not only *Culiseta melanura* but potential bridge vectors. Multiple horse involvement was found in southern New Jersey. Population patterns of the enzootic vector are considered in light of the unusual weather patterns.

P-16 West Nile virus human and mosquito metrics in Illinois from 2002 to 2010

Richard Lampman, richlamp@illinois.edu, Ephantus J. Muturi, Barry Alto and John-Paul Mutebi

Although annual and seasonal variation in transmission of West Nile virus (WNV) is driven by the abundance of infected vectors and their feeding patterns on competent and incidental hosts, these factors are sensitive to many genetic, environmental, and ecological variables. Hyperendemic foci are often used a basis for correlative studies to determine the key factors regulating epidemic WNV transmission; however, sometimes conclusions are contradictory. Our goal was to evaluate the spatial and temporal heterogeneity in typical components of West Nile virus surveillance programs (e.g., human cases, mosquito abundance, number of infected mosquito pools, and *Culex* infection rates). If spatial "hotspots" exhibit considerable variability in transmission parameters within and between season variability, then conclusions from short-term studies may not be valid generalizations. The goal of our study was to describe the spatial and temporal heterogeneity in case numbers, incidence per 100,000, and repeatability of cases in Illinois over a 9-year period at 4 spatial levels (i.e., regions of the state, counties, cities, and zip codes). This study includes years with high (2002, 2005 and 2006), medium (2003, 2004, 2007, and 2010), and

low (2008 and 2009) case counts. The epiweek data provide guidelines for evaluating the intensity of outbreaks and is compared to 2012.

P-17 Hole passage behavior in *Anopheles gambiae*: implications for the field assessment of bed net damage
Jim Sutcliffe, jsutcliffe@trentu.ca, Kathryn Benton and Brian Byrd

Anopheles gambiae (G3) females were individually video-recorded in mesh-covered large (30 mm deep X 170 mm diam) or small (30 mm deep X 85 mm diam) behavioral arenas oriented vertically beside, or horizontally above, the experimenter to simulate the side (vertical surface) or top (horizontal surface) of a human-occupied rectangular bed net. The mesh of each arena had either a 13 mm diam or a 9 mm diam (approx. area ratio=2:1) hole cut in the center to simulate bed net damage. The amount of time spent in flight for each mosquito in the arena was logged using Observer® software and the probability of hole passage per unit flight time (PHP/UFT) was analyzed using a Cox proportional hazards model. We found that: 1) mosquitoes flew significantly more in arenas placed above the experimenter; 2) irrespective of orientation, mosquito PHP/UFT was significantly greater for 13 mm holes; 3) for respective hole sizes, mosquito PHP/UFT was significantly greater in the arenas placed above the experimenter; and 4) PHP/UFT for 13 mm holes was significantly greater than twice that for 9 mm holes in vertically-oriented arenas placed beside the experimenter but not in horizontally-oriented arenas placed above the experimenter. These data are presented within the context of evaluating the efficacy of field deployed bed nets and the importance of recording hole size, shape, and location in such assessments is discussed.

P-18 Catching container *Aedes*: comparison of BG Sentinel traps, mosquito magnets, and sticky ovitraps
Ilia Rochlin, ilia.rochlin@suffolkcountyny.gov, Malgorzata Kawalkowski and Dominick V. Ninivaggi

Biogents Sentinel (BGS) trap has become the standard for the Asian tiger mosquito (*Aedes albopictus*) population surveillance. We compared BGS traps baited with BG-lure and octenol to (a) mosquito magnets (MM) baited with BG-lure and R-octenol, and (b) sticky ovitraps baited with oak infusion. BGS and MM traps were deployed at 3 paired urban locations. Each location also contained sticky ovitraps. Compared to BGS traps, MM collected significantly higher numbers of *Ae. albopictus*, *Ae. japonicus*, and *Ae. vexans*, but slightly fewer *Culex* species. MM provided a representative snapshot of biting *Aedes* species, required minimum maintenance, and could be used to obtain large number of *Aedes* specimens for arbovirus testing. Sticky ovitraps collected low numbers of mostly *Ae. albopictus* and *Ae. japonicus* providing a presence/absence estimates, but were cumbersome to deploy and maintain. We concluded that MM is a viable alternative to BGS traps especially for establishing permanent sites for long-term container *Aedes* surveillance.

P-19 Modeling human cases of West Nile virus in New Jersey
Lisa Reed, lreed@rci.rutgers.edu, Scott Crans, Mark Robson and Randy Gaugler

Since the emergence of West Nile virus in the US, activity in New Jersey has been variable. Human case load per year range from 1 to over 30. New Jersey has active mosquito and arbovirus surveillance in all counties, suggesting that surveillance as well as other factors including climate and socio-demographic factors may combine to explain the number of human cases. But deriving a model has proved to be difficult. A previous multiple regression model developed during the early years of outbreak proved to no longer predict well, particularly when applied to a GIS environment. A second look through GIS technology may provide more insight into explaining human cases in New Jersey. Factors explored to explain 2010 cases include population density, minimum field infection rates of various factions of mosquitoes, spring precipitations and departures from normal and bird infection rates with new variables being examined. The model, as of this writing, current explains 50% of the variability. We present the model (both exploratory and geographically weighted) with pros and cons to model development in explaining human disease in a mosquito abatement environment.

P-20 The use of an in vitro bovine assay to predict the efficacy of mitochondrial Complex I electron transport inhibitor insecticides against adult *Aedes aegypti* (L)
Joshua Matta, jmatta@clarke.com and Edmund J. Norris

Global insecticide resistance hinders adequate control of disease-bearing mosquitoes. It is important to investigate molecules designed for use in other arenas for efficacy against mosquitoes and to develop new methods for testing these compounds. Traditionally, the primary means of establishing insecticide efficacy has been through the conduct of whole organism in vivo bioassays, which can be time-consuming and may produce variable results. Insecticide target-site screens should be considered an important method of determining the potential efficacy of candidates prior to validation with live insects. Four synthetic mitochondrial electron transport inhibiting (METI) insecticides: pyridaben, tebufenpyrad, tolfenpyrad, and fenpyroximate, were target site screened using an enzyme assay prior to in vivo adult mosquito bioassays against *Aedes aegypti* (L). All of the insecticides exhibited similar enzyme activity ranging from 75-88% inhibition of mitochondrial Complex I enzyme and were thus all subject to in vivo bioassay. Topical application bioassays suggested that tolfenpyrad was the best candidate as it demonstrated the lowest LD_{50} value (0.01 µg/mosquito). The in vitro prescreen proved to be a useful tool for detecting METI insecticide activity before proceeding to in vivo adulticide trials.

Education

P-21 The misunderstood mosquito: a science lesson for kindergarten
Eric D. Jackson, ericdj@leeschools.net, Brian Murphy and Neil Wilkinson

Each year, thousands of Lee County kindergartners participate in this science-readiness program as provided through the unique collaboration of the Lee County Mosquito Control District, the Lee County School District, and Florida Gulf Coast University. This lesson uses the attributes of the mosquito to teach kindergartners about the basic characteristics of insects including an insect's life cycle. Students create their own mosquito model and view living mosquito larvae. The program correlates with local school district lesson objectives in life science. Florida Gulf Coast University interns, who earn service-learning requirements toward graduation and gain experience working with partnering local agencies, lead the classroom presentations.

P-22 Development of identification keys to the mosquitoes of eight states in the mid-Atlantic region
Bruce Harrison, skeeterdoc@gmail.com, Charles Sither, Parker Whitt and Brian Byrd

Pictorial dichotomous identification keys to the adult females and 4th instar larvae of mosquitoes found in an 8 state Mid-Atlantic region (PA, DE, MD, WV, VA, NC, SC, and GA) were developed using a vector graphics editor. These keys identify 13 genera and 88 named, provisional, or hybrid species taxa, including certain members of the Crucians and Punctipennis Complexes, and the Quadrimaculatus Group. Biological and taxonomic notes are provided for clarifying possible identification problems, and state distribution tables are also included. A key component of this project was finding additional characters for separating as many included species as possible. These efforts are reflected in numerous multi-character couplets. The benefits and challenges of using vector graphics in Adobe Illustrator® are presented. Plans to disseminate the more than 700 morphological images (*.ai files) through a searchable database are discussed in the context of "open sourcing" the project for other mosquito control and identification professionals.

Equipment

P-23 Evaluation of public health spraying equipment
Wesley C. Hoffmann, clint.hoffmann@ars.usda.gov, Brad Fritz, Todd Walker, Muhammad Farooq and Vincent Smith

Droplet size spectra from different sprayers used to generate sprays for controlling insects that may vector diseases were measured with a laser diffraction instrument. The objective of this work was to measure the spray system droplet size generated by different sprayers with water- and oil-based spray solutions for public health sprayers, such as thermal foggers and ultra-low-volume sprayers. Data from 14 sprayers and different spray solutions showed a wide range in the droplet size spectra produced. The volume median diameter (DV0.5) measurements ranged from 4-94 µm for the public health sprayers evaluated. Several of the citrus sprayers were also evaluated for engine speed adjustments to generate different size droplets. The information presented aids pesticide applicators in sprayer selection and operation to produce the specific droplet size spectra required for a particular application.

Genetics

P-24 Primary isolation of stem cells from midgut epithelium of *Culex pipiens* mosquitoes
Belal A. Soliman, ba.soliman@yahoo.com, M. Wassem, Hala Gabr Metwaly and Mai Ibrahim Hussein

Stem cells are undifferentiated cells that have the remarkable ability to self-renew and produce differentiated cells that support normal development and tissue homeostasis. This unique capacity makes stem cells a powerful tool for future regenerative medicine and gene therapy. The stem cells are critically important in that they are capable of massive proliferation and differentiation. The present protocol describes of primary cell cultures from mosquitoes' larval midgut. Mosquito stem cells have been described from embryonic stage, late 3rd larval stage of *Culex pipiens*. The midgut stem cell niche is comprised of both differentiated columnar and goblet cells as well as hormonal and other signaling molecules in the haemolymph. Out of dissected larvae 100 cells were harvested 24 h post cell culture and after 48 h became 151cells. This finding seems to show that the harvested cells increased in half of its initial number. This means that some mother stem cell may be divided into 2 or more new cells. We examined what stem cell types have been identified from insects and briefly discuss what is known about their regulation. Genetically modified stem cells can be used to produce transformed mosquito.

P-25 Rapid discrimination between *Anopheles gambiae* s.s. and *Anopheles arabiensis* by High-Resolution Melt (HRM) analysis
Michael R. Zianni, Zianni.1@osu.edu, Mahmood R. Nikbakhtzadeh, Bryan T. Jackson, Jenny Panescu and Woodbridge A. Foster

There is a need for more cost-effective options to more accurately discriminate among members of the *Anopheles gambiae* complex, particularly *An. gambiae* and *An. arabiensis*. The method described here, High-Resolution Melt analysis (HRM), takes advantage of minute differences in DNA melting characteristics, depending on the number of incongruent single nucleotide polymorphisms in an intragenic spacer region of the X-chromosome-based ribosomal DNA. The 2 species in question differ by an average of 13 single nucleotide polymorphisms giving

widely divergent melting curves. A real-time PCR system, BioRad CFX96, was used in combination with a double-stranded DNA-specific dye, EvaGreen, to detect and measure the melting properties of the amplicon generated from leg-extracted DNA of selected mosquitoes. Results with 7 individuals from pure colonies of known species, as well as 10 field-captured individuals unambiguously identified by DNA sequencing demonstrated that the method provided a high level of accuracy. The method was used to identify 86 field mosquitoes through the assignment of each to the 2 common clusters with a high degree of certainty. Each cluster was defined by individuals from pure colonies. HRM analysis is simpler to use than other methods and provides comparable or more accurate discrimination between the 2 sibling species, but requires a specialized melt-analysis instrument and software.

Larval Control

P-26 Control of *Culex pipiens* at a California sawmill facility by utilizing the existing irrigation system to distribute Altosid® (methoprene) larvicide
Mary A. Sorensen, marys@placermosquito.org, Kelly Burcham and Joel Buettner

At a sawmill facility where logs are sprinkler irrigated to prevent drying and splitting, *Culex pipiens* populations can reach hundreds or even thousands of females per trap-night (CO_2 baited Fay traps). The mosquito larvicide Altosid (methoprene) was injected into the irrigation main-line, and irrigation water was collected from multiple locations on the property for 1 h after larvicide injection for use in a bioassay test. Laboratory-reared *Cx. quinquefasciatus* larvae failed to emerge as adults from any point on the western half of the property. Eastern locations showed no evidence of treatment.

P-27 Mosquito larvicides from cyanobacteria
Gerald Berry, jerryaberry@aol.com, John Berry and Fernando Noriega

Cyanobacteria ("blue-green algae") produce a diverse array of toxic or otherwise bioactive metabolites. These allelochemicals may also play a role in defense against potential predators and grazers, particularly aquatic invertebrates and their larvae, including mosquitoes. We are investigating the potential for development and application of toxins of cyanobacteria from the Florida Everglades as insecticides against the mosquito *Aedes aegypti*, the vector of dengue fever and yellow fever. Cyanobacteria biomass screening identified several strains of cyanobacteria that exhibited larvicidal activity against mosquito larvae. We are in the process of identifying the chemical structure and biological properties of some of these toxic fractions.

P-28 Using *Mesocyclops* and essential oils from *Cryptomeria japonica* to control dengue mosquito larvae
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The aim of this study is to control mosquito populations by establishing novel mosquito control strategies with better environmental sustainability, higher specificity, and cost effectiveness. In this study, indigenous *Mesocyclops* species with higher predatory efficiency were collected and identified in Chiayi, Tainan, Kaohsiung and Pingtung in southern Taiwan, which has been recognized the areas with recurrent dengue epidemics. Our results demonstrated the consistent predatory efficiency both in the laboratory and in the field. With the limited sensitivity to the currently used pesticide, copepods therefore can be a novel and cost-effective tool for the biological control of disease vectors. Additionally, there have also been novel or newly identified species of copepods in this study. As a potential intermediate host for various parasites, microscopic and molecular detection have also been performed prior to the establishment of the laboratory colonies for the further evaluation as a control strategy of the vector populations. Combination of Taiwanese freshwater *Mesocyclops* spp. and essential oils from black heartwood-type *Cryptomeria japonica* to control dengue mosquito larvae revealed a severe impact on larvae's survival rate. With the successful technique in mass culture of copepods in laboratory and available resource of essential oils from local forest, it suggests using copepods and essential oils to control dengue vector is feasible and valuable in Taiwan.

P-29 High and low tech methods of West Nile virus surveillance and control in Champaign, Ilinois
William J. Blackford, jblackford@c-uphd.org and James Roberts

The Champaign-Urbana Public Health District (CUPHD) has an integrated *Culex* mosquito management program in the cities of Champaign, Urbana and Savoy, Illinois, which utilizes both high tech and low tech methods for surveillance and abatement to reduce West Nile virus (WNV) incidence in these communities. Abatement initiatives include treatment of pre-identified catch-basins utilizing geographic information system (GIS) mapping, a high tech tool, to plan routes and record progress. A low tech green initiative of the program is the deployment of a team of bicyclists to evaluate and treat these catch basins. Surveillance of WNV involves collecting qualifying birds and sending them to the University of Illinois College of Veterinary Medicine for WNV testing. In addition, *Culex* mosquitoes from multiple locations throughout the community are collected with gravid traps and tested using an in-house Rapid Analyte Measurement Platform® test to determine the presence of WNV. Media alerts and education are disseminated to the public based on surveillance data. Implementing an integrated *Culex* mosquito management program that utilizes both spectrums of technology has proven very successful in our community. This methodology can easily be replicated in other communities across the country.

P-30 Simple, novel, inexpensive larval bio-assay unit for evaluation of insect growth regulators applied in the field

Scott C. Crans, scrans@aesop.rutgers.edu, Ralph Strano and Sean Devaney

Increased use of insect growth regulators applied using low volume/ultra low volume methodology with traditional adulticiding equipment has created the need to evaluate spray coverage and efficacy rapidly. Unfortunately, evaluating field application of insect growth regulators often requires additional time and expense to accurately estimate treatment efficacy which can be challenging for small mosquito control programs on a limited budget. An inexpensive larval bio-assay unit is presented here to aid the process, simplify protocols and save staff time.

Latin American

P-31 *Aedes aegypti* infestations in residential areas during the rainy season in Merida, Yucatan, Mexico

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The Mexican city of Merida inYucatan State has one of the highest levels of dengue transmission in Mexico. We report a preliminary analysis of vector infestation in a sample of 20 neighborhoods of entomological and epidemiological importance throughout the city. The study is part of a project to develop innovative community-based ecosystem interventions directed to reduce vector populations and their interaction with humans. A cross-sectional entomological survey was carried out during the wet season 2012 to determine the presence and abundance of oviposition sites and immature (larvae and pupae) as well as adult female *Aedes aegypti* detected indirectly (through ovitraps placed in the peridomestic area) and directly (through collections of indoor resting mosquitoes with back-pack aspirators). Despite the continuous efforts of local health authorities, *Ae. aegypti* breeding is still prolific and diversified at the household level. Overall, the house index was 20% with an average of 11.6 immature mosquitoes/household. Buckets (20 L), diverse small items (<5 L) disposable or useful (i.e., utensils for cooking/washing), tires and large discarded containers were the most productive types of containers, producing altogether 82% of all pupae found. Exposure at the household level to mosquitoes seems very likely. The proportion of houses reporting positive ovitraps was 62%, with an average of 125.5 eggs/positive ovitrap. Indoor adult mosquito collections showed the presence of adult *Ae. aegypti* in 70% of houses with 2.4 females/household. Based on the situation analysis, we are implementing an intervention directed at both immature and adult vector control through a partnership model with: 1) environmental/water management, behavioral interventions and chemical control targeting the most productive containers and 2) the installation of DuraNet™ long-lasting insecticidal nets as door/window screening in houses.

P-32 Network for the surveillance of insecticide resistance in Colombia

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In Colombia, the network for surveillance of insecticide resistance is part of the National Entomology Network. The Network was created in 2005 thanks to a national project that allowed the validation of 2 biological testing methodologies to evaluate the susceptibility of malaria and dengue vectors to insecticides used in public health and biochemical tests to determine possible mechanisms of resistance. For over 6 years it has been possible to assess the susceptibility of *Aedes aegypti* and the 3 primary vectors for malaria (*Anopheles darlingi*, *An. albimanus* and *An. nuneztovari*) in Colombia to insecticides used by public health programs in the priority locations for malaria and dengue. *Aedes aegypti* populations assessed during this period have shown resistance to DDT (100%), lambdacyhalothrin (51%) and temephos (43%). Nine of 38 *Anopheles* populations were resistant, 6 of them were resistant to lambdacyhalothin. The results of this network are used in Colombia for decision-making and purchasing inputs from local and central levels of the vector control programs.

P-33 Differentiation of *Anopheles (Nyssorhynchus) nuneztovari* s.l. populations from three Colombian regions

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Anopheles nuneztovari s.l. Gabaldon is a species complex composed of at least 2 cryptic taxa distributed from northern South America to the Brazilian Amazon, and in Colombia and Venezuela it is a primary vector of malaria. Population genetics studies can provide information about gene flow patterns and population differentiation that may influence vector capacity and behavior. In Colombia, previous studies did not provide evidence of structure between western and northeastern populations; however, the low number of localities and the molecular markers used might have hindered the detection of existing variation. Therefore, the present work evaluated population structure of *An. nuneztovari* s.l. from 8 sites in Colombia. A total of 220 specimens were analyzed using a 1,180 bp fragment of the mitochondrial DNA Cytochrome Oxidase subunit I gene. Molecular variance and F_{ST} analysis showed population structure among western-W, northeastern-NE and eastern-E populations, with the number of migrants among the 3 populations ranging from 0.1 - ∞. In most cases, values of the tests for neutral evolution were negative and non significant. Three divergent clades were detected: Clade I-W, Clade II-NE and Clade III-E. The results detected population structures that may be influenced by barriers such as the Andean mountains that

acted to reduce gene flow among populations. The divergence observed may also indicate differences in demographic history among the 3 clades.

P-34 Hematophagous Diptera collected from ruminant livestock in Xmatkuil, Yucatan, Mexico

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During the rainy season in 2012, hematophagous Diptera were collected with Shannon traps and while landing on the following ruminant species: a domestic goat (*Capra hircus*), a domestic sheep (*Ovis aries*) and a domestic calf (*Bos taurus*) on a farm near Xmatkuil, Yucatan, Mexico. Overall, the most abundant species of hematophagous flies collected were the stable fly (*Stomoxys calcitrans*) followed by the horse fly (*Tabanus occidentalis* var. *dorsovittatus*). Other species found (but less frequently) were the tabanids (*Leucotabanus itzarum* and *Tabanus commixtus*) and the mosquitoes (*Culex quinquefasciatus*, *Aedes aegypti* and *Ae. taeniorhynchus*). The majority (73%) of flies were collected attracted to the calf.

P-35 Characterization of hemocytes of *Rhodnius prolixus* and *Rhodnius robustus*

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The hemocytes are defense cells in insects that have been associated with infections. Their morphology can be related to type, number and potential role in cellular defense and the molting processes. The aim of this study was to differentiate the number and type of hemocytes before and after molting into nymphs of V stage *Rhodnius prolixus* and *R. robustus*. Aliquots of hemolymph of both triatomines in stage IV were observed. The hemocytes were stained with Giemsa, Gomori, and Schiff stains. The same process was performed on nymphs who molted to stage V. The plasmatocytes were most abundant in the nymphal stages IV and V followed by prohemocytes and granulocytes. Other cells types were observed: spherulocytes, adipohemocytes and oenocitoides. There were no statistically significant differences among the main groups of cells: plasmatocytes: 0.1741, prohemocytes p: 0.1320, and granulocytes p: 0.1810 in differential counts. Two lineages of hemocytes were found: plasmatocytes and granulocytes. Plasmatocytes are likely to play an important role in cellular defense for its abundance and emission of pseudopods. Finally, given the presence of prohemocytes in all stages, it is possible that they are the precursor blood cells in these insect species.

P-36 Synthesis and larvicidal activity of phenylethylamine derivatives

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Control of vector-borne diseases is been threatened by the occurrence of insecticide resistance and their management is limited by the availability of very few insecticide molecules for public health. The development of novel insecticides is necessary. Our research group has conducted an extensive search for new biologically active phenylethylamine derivatives. The purpose of this study was to design and synthesize new macrocyclic and lineal phenylethylamines from L-tyrosine and to evaluate their insecticidal activity by bioassays against the malaria vector (*Anopheles albimanus*), the dengue vector (*Aedes aegypti*), and the house mosquito (*Culex quinquefasciatus*). A total of 6 lineal and 2 macrocyclic phenylethylamine derivatives were synthesized. All compounds were evaluated in bioassays in the laboratory, against 3rd and 4th instar larvae of *An. albimanus, Ae. aegypti* and *Cx. quinquefasciatus*. Three replicates were carried out for each species and compound, and DL_{50} values were calculated. A lineal phenylethylamine (4-[2-(acetylamino)ethyl]phenyl acetate) showed the best larvicidal activity against the 3 mosquito species. The analysis of the structure-activity relationship indicates that lineal phenylethylamines are more active than macrocyclic phenylethylamines. The larvicidal activity of lineal phenylethylamines provides further evidence to aid in the search for more potent analogues.

P-37 Comparison between the productivity of *Aedes albopictus* and *Ae. aegypti* pupae in different breeding sites in Medellin, Colombia

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Aedes albopictus is a mosquito species from Asia, where it has been considered as an important vector for dengue viruses. Its ecological flexibility has allowed it to spread to different countries. In Colombia, this invasive species was detected in 1998 and in 2011 was, for the first time, reported in Medellin, the city with the largest human population in Colombia where dengue is endemic. Pupae from natural and artificial habitats were collected between June, 2011 and July, 2012 to compare the pupal productivity and to establish the potential displacement by *Ae. albopictus* of *Ae. aegypti*. Pupae collected in the field were kept in the laboratory until adults emerged. The statistical analysis showed that tires were more productive oviposition sites than bamboo and water plants for *Ae. albopictus* as well as for *Ae. aegypti*. In addition, a temporal fluctuation in the density of both species that alternated between the 2 of them was observed. Preliminary results show a non-displacement of the invading species; however, it is necessary to extend the study in order to confirm these results. These findings improve the entomological understanding of dengue transmission dynamics in Medellin and provides information that is useful for the design of appropriate control strategies.

P-38 Evaluation of susceptibility of *Aedes aegypti* to organophosphates in the municipality of Malambo, Atlantico, Colombia

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Between 1999 and 2010, Malambo in the department (state) of Atlantico in Colombia was ranked as the 9th largest

municipality with endemic dengue transmission in the Colombian Caribbean region with 1,637 reported cases. To control this disease, insecticides have been used on vector populations as the primary measure to control dengue outbreaks or epidemics. However, during the last 3 years, only organophosphate molecules have been applied because *Aedes aegypti* is resistant to pyrethroids. In 2012, we evaluated the susceptibility of a subpopulation of *Ae. aegypti* from Malambo to the following insecticides- temephos, malathion, fenitrothion and pirimiphos methyl. The resistance ratios were calculated from the lethal concentration 50 (LC_{50}) determined for each insecticide in the field subpopulation and compared with the international susceptible reference strain (Rockefeller). In Malambo, the *Ae. aegypti* sub-population had the following resistance ratios (RR) to the organophosphates evaluated: temephos (RR=2x), malathion (RR=3x), fenitrothion (RR=1x), and pirimiphos methyl (RR=3x). We provided these results to the Malambo officials who were applying the insecticides that we evaluated in this field work about dengue control.

P-39 Ectoparasites infesting rural dogs in the municipality of Merida, Yucatan, Mexico

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We present preliminary results of a study on ectoparasites (ticks and fleas) infesting rural dogs from Molas in the municipality of Merida, Yucatan, in southeastern Mexico. One hundred fifty dogs from this rural community were examined. Ticks were found on 88.6% of the dogs and 6 tick species were identified. The most common species were *Rhipicephalus sanguineus* (62.7%), *Amblyomma inornatum* (20.3%), and *Dermacentor variabilis* (15.21%). Other species found were *A. cajennense*, *A. ovale*, *Ixodes scapularis* and *Haemaphysalis* sp. Fleas were found on 74% of the dogs and 3 flea species were identified: *Ctenocephalides felis*, *Ct. canis* and *Echidnophaga gallinacea*. Overall, the highest frequency of ectoparasites was found on the head (61%) of the dogs. Previous studies in urban and some rural areas of the municipality have reported variable infestation levels, but normally lower species richness. The diversity of ectoparasities found infesting dogs from Molas may be explained by the life style of dogs (i.e., living in the backyard with other animal species, semi-domiciled and with free access to surrounding forested areas) and, therefore, more exposed to ectoparasites.

P-40 Sand fly species associated with an outbreak of cutaneous leishmaniasis in the department of La Guajira, Colombia

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Cutaneous leishmaniasis is endemic in the department (state) of La Guajira, Colombia. During epidemiological week 36 of 2011, 6 cases of cutaneous leishmaniasis were reported and confirmed from the area of Carreipia in the municipality of Maicao. Prior to 2011, this area of the department had never documented an outbreak of this disease. For this reason, an entomological survey was conducted in the possible areas of infection to identify the sand flies species that might be involved in disease transmission. Collections were made with CDC light traps during 3 consecutive nights in 6 houses in the rural area of Carreipia, including the intra- and peridomiciliary areas. Collected sand flies were preserved in 70% alcohol, rinsed with potassium hydroxide and 10% liquid phenol and examined under a microscope with the Young and Duncan key to determine the species present. A total of 226 sand flies were collected belonging to 2 species of the subgenera *Lutzomyia Franca* and *Psychodopygus*. The species recorded were *L. panamensis* (70.3%) and *L. gomezi* (29.6%). *Lutzomyia panamensis* was found in 100% of the households and around 67% of the homes, while *L. gomezi* was only found around 33% of the homes sampled. This records the presence of *L. panamensis* and *L. gomezi* as possibly involved in the transmission of leishmaniasis in Colombia. The discovery of these species and their possible association with houses allows us to orient control measures in order to control future outbreaks.

P-41 Wing geometric morphometrics as a tool for the differentiation of Colombian *Anopheles* species of two subgenera

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From approximately 47 *Anopheles* species registered in Colombia, only 9 have been incriminated as malaria vectors. Morphological identification of some species is problematic because of intraspecific phenotypic variation and interspecies similarity. Wing geometric morphometrics appears as an alternative, low-cost, fast and promising tool for taxonomy. In this study, wing size and shape variations were analyzed for species of 2 subgenera, *Anopheles* (7) and *Kerteszia* (1). A total of 119 wings were photographed and 13 landmarks digitized. Variance analysis showed significant differences in wing isometric size and the pairwise comparisons detected some differences among species. Specimens of *An. (Anopheles) costai* showed the larger wing sizes and *An. (Kerteszia) neivai* the smallest. Principal component analysis and shape significance test revealed wing shape differences among some species. Canonical variate analysis (CVA) performed as a multigroup discriminant function using the wing shape variables allowed the assignation of each specimen to a given group. CVA showed an excellent discrimination of *An. neivai* and variable for the *Anopheles* subgenus species, but in general, the level of discrimination was in agreement with morphological identification. The results indicate that morphometrics is a useful tool for the discrimination of the species evaluated and it could contribute to entomological surveillance.

P-42 Insecticidal activity of Girgensohnina structural analogues on *Aedes aegypti* larvae: A study of mitochondrial bioenergetics and apoptosis

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Girgensohnina alkaloid analogues, obtained through Strecker reaction SSA catalyzed, act as insecticides on *Aedes aegypti* larvae. Insecticidal activity, bioenergetics mitochondrial and release of cytochrome C was evaluated using analogues on *Ae. aegypti* larvae. The larvae were between the 3rd and 4th instar and insecticidal activity was determined by lethal concentrations (LC_{50} and LC_{99}) using Probit analysis. Mitochondria were isolated by differential centrifugation. Type Clark Oxygraph was used to determine the respiratory control coefficient (RCC ≥3) as indicating integrity of mitochondria. The effect of the most promising analogue was analyzed on protein complexes I and II of the electron transport chain of mitochondria. The release of cytochrome C was evaluated as an indication of apoptosis with a 6100PC UV spectrophotometer at 550 nm. All analogs had larvicidal activity (24 h LC_{50} = 88.12 ppm and LC_{99} = 147.54 ppm). At mitochondrial level was found that a concentration of 2E-03 ppm inhibits cellular respiration pathway complex I and II in independent dosage form. It was observed that the release of cytochrome C in energized mitochondria such as in non-energized. We conclude that the analogs evaluated affect mitochondrial bioenergetics and generate the apoptosis process in *Ae. aegypti* mitochondria.

P-43 Field evaluation of lethal ovitraps for the control of *Aedes aegypti* in Medellin, Colombia

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In Colombia, dengue is the major vector-borne disease as a result of the large human population exposed and the disease's lethality. The absence of a vaccine for dengue prevention has led to development of alternative strategies focused on effective vector control. For this reason, different types of insecticides, attractants and oviposition substrates were selected under laboratory conditions, and led to the evaluation in the field of 6 lethal ovitraps (LO). The LO configurations selected were: 1. deltamethrin/10% hay infusion/paper towel, 2. deltamethrin/water/paper towel, 3. deltamethrin/water/dacron, 4. deltamethrin/10% hay infusion/flannel, 5. deltamethrin/20% hay infusion/flannel, and 6. deltamethrin/water/flannel. In an area of high dengue transmission in Medellin, 30 households were randomly selected (5 houses for each type of LO), in order to evaluate each LO with its respective control trap. Traps were checked every 7 days for 30 days, recording the number of eggs laid and the hatching rate. A statistically significant difference was observed in the number of eggs laid and hatched in different LOs, with the combination deltamethrin/10% hay infusion/paper towel LO yielding the best results. This information may be used by city health authorities to help them develop a better strategy for surveillance and control of dengue in Medellin.

P-44 Insecticidal activity, mitochondrial bioenergetics and apoptosis evidence generated by essential oils of *Eucalyptus citriodora* (Myrtaceae) and *Cymbopogon flexuosus* (Poaceae) on *Aedes aegypti* larvae (Diptera: Culicidae)

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Essential oils (EO) are a source of new insecticides. The insecticidal activity, mitochondrial bioenergetics and release of Cytochrome C were evaluated when *Cymbopogon flexuosus* and *Eucalyptus citriodora* EOs were applied to *Aedes aegypti* larvae. We used larvae between the 3rd and 4th instar and insecticidal activity was determined as lethal concentrations (LC_{50} and LC_{99}) using Probit analysis. Mitochondria were isolated by differential centrifugation. Type Clark Oxygraph was used to determine the respiratory control coefficient (RCC ≥3) as indicating integrity of mitochondria. The effect of EO on protein complexes I and II of the electron transport chain of mitochondria was evaluated. The release of Cytochrome C was analyzed as an indication of apoptosis with a 6100PC UV-spectrophotometer at 550 nm. The results indicated that oils had larvicidal action (*C. flexuosus*, IC_{50}=5 ppm and LC_{99}=56 ppm and *E. citriodora*, IC_{50}=51ppm and LC_{99}=724 ppm). *Cymbopogon flexuosus* affects RCC at ≥5ppm when electrons are derived from the complex I. *Eucalyptus citriodora* at ≥20 ppm affects the electron transport from the complex II. Cytochrome C release from mitochondria both energized and non-energized was observed. We conclude that the *C. flexuosus* and *E. citriodora* EOs have insecticidal activity, affect mitochondrial bioenergetics and generate the apoptotic process in *Ae. aegypti* mitochondria.

P-45 The importance of street storm sewers for the production of adult *Aedes aegypti* during the dry season in a neighborhood of Merida, Yucatan, Mexico

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We recently reported for the first time immature *Aedes aegypti* in underground storm sewers of the streets in the city of Merida, Yucatan state in southern Mexico, an endemic area for dengue transmission. This study was conducted during the 2011 rainy season and showed high levels of *Ae. aegypti* positivity (presence of larvae and/or pupae) in these non-residential habitats, but no estimation of adult productivity was made. We compared the number of adult *Ae. aegypti* mosquitoes emerging from storm sewers located on the streets in a neighborhood of Merida against the number of pupae (as a proxy of adults emerging) from all oviposition sites in the premises within 1 ha during the 2012 dry season. Only 7 containers with water were identified from 30 premises inspected, but no pupa-positive containers were found. In contrast, *Ae. aegypti* adults were collected from 60% of the storm sewers. They produced 12 adults per day/Ha (95% CI, 6.4 to 17.9), 5 females (95% CI, 2.1 to 7.7) and 7 males

(95% CI, 3.8 to 10.7). This study reveals the importance of this type of non-residential and subterranean oviposition site in this neighborhood of Merida during the dry season.

Management/Public Relations

P-46 West Nile virus epizootics in vector species of New York City in 2012
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In 2012, the Department of Health and Mental Hygiene conducted West Nile (WN) virus surveillance at 52 permanent and 242 supplementary mosquito trapping sites in the City of New York. Mosquitoes were collected on a weekly basis from the permanent traps from June through October. The supplementary traps were placed to enhance virus surveillance in the areas where WN virus activity was recently identified in mosquitoes, humans or both. A total of 118,890 adult mosquitoes belonging to 29 species were identified, of which 86,134 belonging to 19 species were tested for the presence of WN virus infection. Of the 4,265 mosquito pools tested, 310 were tested positive for WN virus. In 2012, 3 mosquito species, *Culex pipiens*, *Cx. restuans*, and *Cx. salinarius* were infected with WN virus. This year, WN virus arrived earlier than usual; we collected the first WN virus infected pool (*Cx. pipiens*) on June 28 from New Dorp Beach in Staten Island. This season, 21 separate spray (adulticiding) events were conducted over approximately 55,000 acres of residential and natural areas. This season, 25 cases of WN neuroinvasive disease and 12 cases of WN fever were detected in the city. All 37 cases of WN viral disease possibly or probably became infected locally.

P-47 Learning from the opposition: using grassroots strategies for NPDES outreach and education—a case study in California
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New NPDES regulations administered by the California State Water Resources Board (CSWRCB) would significantly affect how mosquito control is conducted in California (CA). A strategy was needed to inform residents and legislators about the potential impacts on public health. Research was conducted on the communication strategies that impact knowledge and attitudes and consequently, policy. Representatives from 4 CA mosquito control districts immediately affected by the regulations held a series of planning sessions to learn the fundamentals of a direct action campaign and identify the tools needed to implement it. Tools developed by the group were implemented to varying degrees across CA, media and community advocacy resulted in meetings with local and federal legislators, and a regulatory affairs committee was formed by Mosquito and Vector Control Association of CA (MVCAC). The process reopened dialogue between the CSWRCB and MVCAC, and revisions to the CA NPDES permit were implemented before a statewide campaign was launched. By utilizing a strategy that has yielded positive results for anti-pesticide groups in educating public officials and influencing public opinion, districts in CA have been able to gain some relief from the CA NPDES permit. This model can be replicated in states seeking similar relief when new regulatory burdens threaten to affect the ability to implement responsible mosquito control and consequently, the ability to protect public health.

Systematics

P-48 Mosquito diversity in New York City
Waheed I. Bajwa, wbajwa@health.nyc.gov, Zahir Shah and Liyang Zhou

Intensive mosquito surveys of New York City were undertaken from 2000 - 2012 to determine mosquito density, distribution and abundance in the city. In this paper, we will compare results of these surveys with similar surveys conducted in 1936, 1950 and 1969 in the city. In these surveys, mosquitoes were collected in light traps. In the recent surveys (2000-2012), we identified 10 genera and 51 mosquito species - *Aedes* (3), *Anopheles* (7), *Coquillettidia* (1), *Culex* (5), *Culiseta* (4), *Ochlerotatus* (23), *Orthopodomyia* (1), *Psorophora* (4), *Toxorhynchites* (2), and *Uranotaenia* (1). In addition, larval sites for most species were identified within the city. In 1936, 27 species belonging to 9 genera were identified. In 1950 and 1969, 37 species belonging to the same 9 genera were found. Two recent introductions to NYC were *Ae. albopictus* and *Oc. japonicus*. *Ae. albopictus* has become well established and is becoming a dominant pest in the city. The results of our study indicate a significantly greater diversity among genus *Ochlerotatus* - 10 species in 1936 to 23 species in the recent surveys.

P-49 Description of four common container-ovipositing mosquito eggs using scanning electron microscopy and automontage microscopy
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The eggs of *Aedes triseriatus* (Say), *Ae. japonicus* (Theobald), *Ae. albopictus* (Skuse), and *Ae. aegypti* (Linnaeus) are described with the aid of color images obtained from an automontage camera system and environmental scanning electron micrographs. All eggs are cigar shaped. The egg of *Ae. triseriatus* is light grey in color with distinct chorionic cells. *Ae. japonicas* is dark grey in color with distinct irregular chorionic cells. *Aedes albopictus* and *Ae. aegypti* are both jet black in color and are differentiated by the presence of a distinct micropylar collar in *Ae. aegypti*. Color images may be useful in identifying field-collected eggs where these species' geographic ranges overlap.

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Zavortink, Thomas	Davis University, Davis, California, United States	139
Zazra, David	North Shore MAD, Northfield, Illinois, United States	119
Zhou, Liyang	NYC Health Department, New York, New York, United States	P-46, P-48, 177
Zianni, Michael	The Ohio State University - Plant-Microbe Genomics Facility, Columbus, Ohio, United States	P-25
Zink, Steven	New York State Dept Health, Albany, New York, United States	228
Zipa, Yaneth	Grupo de Enfermedades Transmitidas por Vectores, Secretaría de Salud de Boyacá, Bogotá, Colombia	134
Zuharah, Wan Fatma	Universiti Sains Malaysia, Minden, Penang, Malaysia	191
Zuzworsky, John	New York City Department of Health and Mental Hygiene, New York, New York, United States	98

AMCA AWARDS

HONORARY MEMBERS

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

HAROLD FARNSWORTH GRAY MEMORIAL CITATION
MERITORIOUS SERVICE TO MOSQUITO CONTROL AWARD

This now discontinued award was presented to an active member of AMCA for exceptional service to the Association and to mosquito control or related vector control.

1964 Fred C. Bishopp (DC)

DR. THOMAS J. HEADLEE MEMORIAL AWARD

This now discontinued award recognizes a living member of the Association for outstanding service to the field of mosquito control, while simultaneously commemorating the name of a deceased member.

1968 George H. Bradley (USDA/USPHS)

MEDAL OF HONOR

Next to honorary membership, the Medal of Honor is the highest award regularly given by AMCA. The only specific limitation for the Medal of Honor is AMCA membership, and nominees are selected on the basis of exceptional contributions to mosquito control or related fields. After 1982, the Board of Directors set a suggested maximum of one Medal of Honor per year.

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

MERITORIOUS SERVICE AWARD

Given to individuals for outstanding service, the contributions of the nominees must be considered outstanding as judged by their peers. Only AMCA members in good standing who are not past presidents of AMCA are eligible. After 1982, the Board of Directors set a suggested maximum of no more than two awards per year.

1972	Charles F. Scheel (IL)
	Donald L. Collins (NY)
	Theodore G. Raley (CA)
1973	Francis P. Creadon (CA)
	Vernon Conant (NJ)
	Austin W. Morrill, Jr. (CA)
1974	Leslie D. Beadle (USPHS)
	John H. Brawley (CA)
	John W. Kilpatrick (GA)
	T. Oscar Fultz (GA)
	Howard R. Greenfield (CA)
	Paul J. Hunt (FL)
	William C. McDuffie (USDA)
	Donald R. Johnson (GA)
	Helen Sollers-Riedel (DC)
1975	Lewis E. Fronk (UT)
	Joseph G. McWilliams (USN)
	Lewis J. Ogden (USPHS)
	Rajindar M. Pal (WHO)
	Kenneth D. Quarterman (USPHS)
	Herbert F. Schoof (USPHS)
1976	Robert A. Armstrong (MA)
	Osmond P. Breland (TX)
	George B. Craig, Jr. (IN)
	Claude M. Gjullin (USDA)
	T. Wayne Miller (FL)
1976	Donald J. Pletsch (Mexico)
	Glenn M. Stokes (LA)
	Luis M. Vargas (Mexico)
1978	Richard C. Axtell (NC)
197	Marco. E. C. Giglioli (BWI)
198	James D. Gorman (FL)
	Donald E. Weidhaas (FL)
	E. John Beidler (FL)
	Eugene J. Gerberg (MD)
198	A. Ralph Barr (CA)
	Gilbert L. Challet (CA)
	Edgar A. Smith (VA)
198	Hugo A. Jamnback (NY)
	Donald R. Johnson (GA)
	Harold D. Newsome (MI)
	James V. Smith (GA)
198	Richard F. Darsie (CO)
	Ronald A. Ward (DC)
198	Samuel G. Breeland (FL)
	Donald J. Sutherland (NJ)
198	John C. Kuschke (NJ)
	James R. Caton (CA)
198	C. Lamar Meek (LA)
198	John C. Combs (CA)
198	Chester G. Moore (CO)
	Margaret Parsons (OH)
198	John S. Billodeaux (LA)
	Edgar S. Bordes, Jr. (LA)
199	Richard D. Morton (WA)
	Lucas G. Terracina (LA)
199	David A. Dame (FL)
199	Jerry Mix (TX)
199	William E. Hazeltine (CA)
199	Sally A. Wagner (MI)
1995	Frederick W. Wagner (KY)
1996	Donald J. Sutherland (NJ)
	Ronald A. Ward (MD)
1997	Roger S. Nasci (CO)
	Thomas J. Zavortink (CA)
1998	James D. Long (TX)
1999	Hilton B. Munns (CA)
2000	Leroy J. Bohn (VA)
	Dreda McCreary (VA)
2001	Charles T. Palmisano (LA)
2002	Thomas G. Floore (FL)
	Sherry McLaughlin (TX)
2003	Wayne L. Kramer (NE)
	John L. Clarke, Jr. (IL)
2004	Yadira N. Rangel (Venezuela)
	James W. Robinson (FL)
2005	Major S. Dhillon (CA)
	William H. Meredith (DE)
2006	William J. Sames (WA)
2007	Henry R. Rupp (NJ)
2008	Allan Inman (CA)
	Manuel Lluberas (FL)
2009	Joe Conlon (FL)
2010	Norbert Becker (Germany)
2011	Harry Savage (CO)
	L.A. Williams (SC)
2012	Lal S. Mian (CA)
	Edsel M. Fussell (FL)

PRESIDENTIAL CITATION

The Presidential Citation recognizes individuals not eligible to receive other awards but who are eminently deserving of special recognition by AMCA. Recipients need not be AMCA members. After 1982 the Board of Directors set a suggested maximum of no more than 2 awards per year.

1980	John M. Poché (LA)
	Leslie E. Fronk (UT)
	Jesse B. Leslie (NJ)
1981	Linda G. Raiche (CA)
	Margaret S. Slater (NY)
1982	K. G. Nolan (NY)
	Charles F. Scheel (IL)
1983	Coyle E. Knowles (NY)
1984	Ray Treichler (DC)
1985	Lawrence T. Cowper (USAID)
	Janice B. Wells (NY)
1986	T. Oscar Fultz (GA)
1987	Sharon A. Colvin (IL)
1988	Daniel D. Sprenger (TX)
1989	Fred C. Roberts (CA)
1990	Leonard E. Munsterman (IN)
1991	James D. Long (TX)
1992	Charlie D. Morris (FL)
1993	Robert J. Novak (IL)
1994	James W. Robinson (FL)
	Dan L. Ariaz (NV)
1995	Sally Kuzenski (LA)
1996	Carl R. Tanner (IL)
	Sammie L. Dickson (UT)
1997	Charles T. Palmisano (LA)
	George J. Wichterman (FL)
1998	Douglas B. Carlson (FL)
1999	Charles Beesley (CA)
	Donald R. Johnson (GA)
2000	Peter B. Ghormley (CA)
	David A. Brown (CA)
2001	Donald Menard (LA)
	Joel Margalit (Israel)
2002	Dennis Moore (FL)
	Henry R. Rupp (NJ)
2003	James R. McNelly (NJ)
	Robert Bonnett (MN)
2004	James R. Brown (FL)
2005	Mark Newberg (IL)
	Susan Maggy (CA)
2006	Teung Chin
2007	Karl Malamud-Roam (CA)
2008	Wiliam H. Meredeth (DE)
2009	Rep. Dennis Cardoza (CA)
2010	Gordon Patterson (FL)
	Gary Clark (FL)
	Yasmin Rubio-Palis (Venezuela)
2011	Angela Beehler (WA)
	Roxanne Connelly (FL)
2012	Truc Dever (CA)

JOHN N. BELKIN AWARD

The John N. Belkin Award is given for meritorious contributions to the field of mosquito systematics and/or biology and may be given to anyone judged by his peers to be worthy. Usually, a maximum of one award per year is given.

1981	Botha de Meillon (PA)
1982	Lloyd E. Rozeboom (IL)
1983	Kenneth L. Knight (NC)
1984	Thomas J. Zavortink (CA)
1985	Stanley J. Carpenter (CA)
1986	Elizabeth P. Marks & John Reid (Australia)
1987	James B. Kitzmiller (FL)
1988	Allan R Stone (MD)
1989	Pedro Galindo (Panama)
1990	Peter F. Mattingly (UK)
1991	Jose P. Duret (Argentina)
1992	Bruce A. Harrison (NC)
1993	Edward L. Peyton (DC)
1994	Theodore H. G. Aitken (CT)
1995	Oswaldo P. Forattini (Brazil)
1996	A. Ralph Barr (CA) Michael W. Service (UK)
1997	Christine J. Dahl (Sweden)
1998	Ralph E. Harbach (UK)
1999	Yiau-Min Huang (DC)
2000	Lewis T. Nielsen (UT)
2001	John F. Reinert (FL)
2002	Richard F. Darsie (FL)
2003	Richard C. Wilkerson (MD)
2004	Kazua Tanaka (Japan)
2005	Ronald A. Ward (MD)
2006	William K. Reisen (CA)
2008	Maria-Anice Sallum (Brazil)
2010	Daniel Strickman (MD)
2011	Rampa Rattanarithikul, Ph.D. (Thailand)
2012	Maureen Coetzee, Ph. D. (South Africa)

MEMORIAL LECTURE HONOREE & MEMORIAL LECTURER AWARD

The Memorial Lecture Honoree must be one who has made exceptional contributions to the broad field of mosquito control during his lifetime. If there is more than one honoree in a given year, then the group must have made significant contributions as a team or equal stature in the same time frame and to the same aspect of mosquito control. The Memorial Lecturer Award is given to an outstanding speaker (one per year) to present the annual Memorial Lecture in honor of the Memorial Lecture Honoree. The Memorial Lecture Award is not limited to a member of AMCA, but the recipient should be a recognized authority in the broad field of mosquito control.

	HONOREE	LECTURER	TOPIC
1979	Don M. Rees	J. David Gillett	Out for blood: Flight orientation upwind & in the absence of visual clues
1980	Maurice W. Provost	Anthony W. A. Brown	What have insecticides done for us?
1981	Leland O. Howard	Leonard J. Bruce-Chwatt	Leland Ossian Howard (1857-1950) and malaria control then and now
1982	Carlos Finlay Walter Reed William Gorgas Fred Soper	William C. Reeves	A memorial to Finlay, Reed, Gorgas and Soper as major contributors to present-day concepts essential for control of mosquito-borne viruses
1983	Harry H. Stage	Michael W. Service	Biological control of mosquitoes—Has it a future?
1984	Louis L. Williams	George B. Craig, Jr.	Man-made human disease problems: Tires & LaCrosse virus
1985	Thomas J. Headlee	William R. Horsfall	Mosquito abatement in a changing world
1986	Marston Bates	A. Ralph Barr	The basis of mosquito systematics
1987	William B. Herms Harold F. Gray	Robert K. Washino	
1988	John A. Mulrennan, Sr.	Susan B. McIver	Mosquitoes, medicine & memories
1989	Brian Hocking	John D. Edman	Are biting flies gourmet or gourmand?
1990	John N. Belkin	Thomas J. Zavortink	Classical taxonomy of mosquitoes—A memorial to John N. Belkin
1991	Edward S. Hathaway Anderson B. Ritter	C. Lamar Meek	Les maringouins du mech: The legacy of two men
1992	Sir Patrick Manson	Bruce F. Eldridge	The man we honor
1993	Willard V. King	Ronald A. Ward	Renaissance man of medical entomology
1994	Stanley B. Freeman	Mir S. Mulla	Now & in the future
1995	Maurice T. James	Wayne A. Rowley	Maurice T. James
1996	Telford H. Work	Charles A. Calisher	Telford H. Work—A tribute
1997	Stanley J. Carpenter	Lewis T. Nielsen	In honor of Stanley Carpenter
1998	George B. Craig, Jr.	Robert J. Novak	George Brownlee Craig
1999	A. Ralph Barr	Andrew J. Spielman	
2000	John B. Smith	Wayne J. Crans	
2001	William R. Horsfall	Jimmy K. Olson	
2002	Edward F. Knipling	Waldemar Klassen	Titan and Driving Force in Ecologically Selective Area-Wide Pest Management

MEMORIAL LECTURE HONOREE & MEMORIAL LECTURER AWARD (continued)

	HONOREE	LECTURER	TOPIC
2003	Kenneth L. Knight	Ralph E. Harbach	Mosquito systematics: From organism to molecules—A tribute to Kenneth L. Knight
2004	Donald J. Pletsch	David A. Dame	Six Decades of International Commitment
2005	William E. Hazeltine	Bruce F. Eldridge	William E. Hazeltine: Rebel with a cause
2006	William C. Reeves	Grant R. Campbell	
2007	Norman G. Gratz	Graham B. White	Remembering Norman Gratz (1925-2005) – Doyen of Vector Control
2008	Andrew Spielman	John D. Edman	
2009	Lamar Meek	Roxanne Connelly	
2010	Harold C. Chapman	Tokuo Fukuda	
2011	H.G. Dyar	Terry Klein	
2012	James D. Long	John Welch	

INDUSTRY AWARD

Established in 1997, the Industry Award is presented to a representative of a mosquito/vector-related industry who has through his/her efforts advanced the work of mosquito and/or vector control or research.

1997	Charles T. Galley (FL)	2004	John L. Clarke, Jr. (IL)
1998	William German (FL)	2005	Ernest Danka (IL)
1999	Gary A. Mount (FL)	2006	Willie N. Cox (IL)
	Daniel F. Boyd (GA)	2007	Bob Bonnett (MN)
	David W. Waldron (GA)	2009	Clarke Hudson (IL)
	J. David Waldron (GA)		Bill Strange (ID)
2002	Robert E. Richard (TX)	2010	Peter Connelly (FL)
2003	Allen W. Wooldridge (FL)	2011	David Sullivan (MT)
		2012	Stephanie Whitman (WY)

GRASSROOTS AWARD

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

2005	Omar S. Akbari	Reno Washoe Country, Nevada
	Christopher Trapp	Multnomah County Vector Control,
2006	John Phelps	Mercer County, New Jersey
2008	Chris Frame	Cape May County, New Jersey
2009	Jason Craig Hardman	Salt Lake City MAD, Utah
2010	Jessica Fales	Midland County MC, Michigan
	Gary Hillsdale	Metropolitan MCD, Minnesota
	Elizabeth Vice	Buttle County MVCD, California
2011	David Bruget	Kings MAD, California
	Russell Eck	Washoe County Health District, Nevada
	Phillip Henry	Butte County MVCD, California
	Levi Zahn	Williston VCD, North Dakota
2012	Mike Smith	Anastasia MCD, Florida

STUDENT PAPER COMPETITION AWARDS

The AMCA Student Competition was established in 1988 to recognize the outstanding student research paper presented at the annual meeting. Judging of oral presentations is based upon organization, delivery, clarity and effective use of visual aids. In 1991, a $500 cash award was presented to the winner, and in 1998 the Hollandsworth Prize was established by the family of Gerald Hollandsworth to encourage student participation in the AMCA national meeting. There is a $250 prize for honorable mention.

1989	Scott Willis	McNeese State U.	2005	Wesley Rubio	San Diego State U.
1990	Andrea Brown	Peru State Coll.		Whitney Qualls*	Auburn University
1991	John Paul Mutebi	Notre Dame U.		Rebecca Trout*	University of Kentucky
1992	Rosmarie Kelly	U. Massachusetts	2006	Robert D. Anderson	University of Winnipeg
1993	Merry L. Holliday-Hanson	U. California, Davis		Linda O'Connor**	University of Delaware
1994	John E. Gimnig	U. California, Davis		Joshua R. Ogawa*	Oregon State University
	Alice Shaeffer*	U. Mainz, Germany		Matthew Eaton*	Concordia College
1995	Glen Scoles	Notre Dame U.		Linda M. Styer*	U. California, Davis
	Jittawadee Rochaeroen*	U. California, Riverside			
1996	Esther Chow Schaeffer	U. Maryland			

Continued on next page

STUDENT PAPER COMPETITION AWARDS

1997	Lynn Cooper	U. Maryland
1998	C. Roxanne Rutledge	Louisiana State U.
	Emmalee Kennedy*	U. Illinois
	Timothy Schaub*	U. Illinois
1999	Laura Harrington	U. Massachusetts
	Adam S. Jones*	U. Massachusetts
	Hillary Reno*	U. Illinois
2000	Jason L. Rasgon	U. California, Davis
	Hope Q. Liu*	Virginia Polytechnic
2001	No competition	
2002	Laura B. Goddard	U. California, Davis
	Sharon L. Minnick*	U. California, Davis
	Margaret Sherriffs*	Yale U.
2003	Sarah Yaremych	U. Illinois
	Laura Goddard*	U. California
	Jason L. Rasgon*	U. California, Davis
2004	Gregory M. Williams	U. Delaware
	Stephen Aspen*	Colorado State U.
	Christian Kaufmann*	U. Zurich
2007	Jennifer Armistead	University of Florida
	Robert D. Anderson*	University of Delaware
	Thomas M. Mascari*	Louisiana State U.
2008	Jerome Schleier	Montana State University
	Christopher Barker*	U. California, Davis
	Lisa Reimer*	U. California, Davis
2009	Alexandra Chaskopoulou	University of Florida
	Stephanie Larick*	University of Florida
2010	Sarah Wheeler	University of California,
	Kimmy Mains*	University of Kentucky
	Holly Tuten*	Clemson University
2011	Logan Minter	University of Kentucky
	Kristen Meckel-Parker*	San Diego County Vector
2012	Jerome Schleier	Montana State University
	Elizabeth Andrews*	University of Kentucky
	Jennifer Gordon*	University of Kentucky
	Joseph Iberg*	University of Georgia

* - Honorable Mention ** - First Runner Up

AMCA OFFICERS, EXECUTIVE DIRECTORS AND EDITORS

AMCA PRESIDENTS

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

* - Eastern Association of Mosquito Control Workers

AMCA TREASURERS

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

* - Eastern Association of Mosquito Control Workers

SECRETARY, EXECUTIVE SECRETARY, EXECUTIVE DIRECTOR

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

* - Eastern Association of Mosquito Control Workers

BUSINESS MANAGER

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

TECHNICAL ADVISOR

2000-present	Joseph M. Conlon

EDITORS OF *JOURNAL OF AMCA**

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

* - *Mosquito News* became the *Journal* of AMCA in 1985

† - Publication of the Eastern Association of Mosquito Control Workers

‡ - Volume 4, Number 1, was edited by the Publications Committee; subsequent volumes had a single editor

EDITORS OF *MOSQUITO SYSTEMATICS**

1969-1979	Kenneth L. Knight
1979-1992	Lewis T. Nielsen
1992-1993	Lewis T. Nielsen & Ralph E. Harbach, co-editors
1993-1995†	Thomas J. Zavortink, editor, & Lewis T. Nielsen, editor emeritus

* - Prior to 1973 *Mosquito Systematics* was named *Mosquito Systematics Newsletter*

† - In 1995 this publication was discontinued