MOSQUITO VECTOR BIOLOGY AND CONTROL IN LATIN AMERICA—
A 21ST SYMPOSIUM

GARY G. CLARK1 AND YASMIN RUBIO-PALIS2

ABSTRACT. The 21st Annual Latin American Symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 77th Annual Meeting in Anaheim, CA, in March 2011. The principal objective, as for the previous 20 symposia, was to promote participation in the AMCA by vector control specialists, public health workers, and academicians from Latin America. This publication includes summaries of 55 presentations that were given orally in Spanish or presented as posters by participants from 4 countries in Latin America and the USA. Topics addressed in the symposium included: surveillance, chemical and biological control, and insecticide resistance associated with Aedes aegypti; distribution, behavior, and control of Culex; bionomics, ecology, and chemical and biological control of Anopheles vectors of malaria; insecticide resistance; and studies of dengue, West Nile virus, and Triatoma spp.

KEY WORDS Mosquitoes, dengue, malaria, surveillance, control, insecticide resistance

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes, other arthropods, and vectors, and promotes cooperation and interaction among professionals and students in this field both in the USA and internationally. To promote greater and more active participation among and with a portion of its international membership, a Spanish language symposium was held first at the AMCA Annual Meeting in 1991 and at all subsequent meetings. In addition to providing a forum for scientists whose first language is Spanish, the session promotes interaction with mosquito control industry representatives; and interaction with professional colleagues in the USA who are involved in mosquito vector control, training, and research at the university level, and with local, state, and federal government officials.

This publication includes summaries of 55 presentations that were given in Spanish by participants from 4 countries in Latin America, the United Kingdom, and the USA. Topics addressed in the symposium included surveillance, chemical and biological control, and insecticide resistance associated with Aedes aegypti (L.) and dengue viruses; distribution, behavior, and transmission of West Nile virus, and control of Culex; bionomics, ecology, and chemical and biological control of Anopheles vectors of malaria; insecticide resistance; and studies of Triatoma spp. Summaries of 18 previous symposia have been published (Clark and Suarez 1991, 1992, 1993; Clark 1995, 1996; Clark and Rangel 1997, 1998, 1999; Clark et al. 2000; Clark and Quiroz-Martinez 2001, 2002, 2004, 2005; Clark and Rubio-Palis 2006, 2007, 2008, 2009, 2010).

SUMMARIES

Why is vector control not controlling dengue?

Roberto Barrera
Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico

Because the incidence of dengue and dengue hemorrhagic fever continues increasing in the Americas, it is necessary to evaluate why vector control is not controlling dengue. The fact that Aedes aegypti was eliminated from most of the Americas several decades ago makes it difficult to understand why current vector control programs cannot keep Ae. aegypti below vector densities to prevent dengue virus transmission. The main tools used to eliminate Ae. aegypti were “focal application” of residual insecticides in and around water containers (e.g., DDT), source reduction, and a disciplined, vertically structured control program. Most common current approaches to control Ae. aegypti and prevent dengue consist of house inspections for source reduction and larviciding, space spraying of insecticides from vehicle-mounted equipment, and perifocal control around dengue cases. These approaches fail at delivering the controlling agent to a significant portion of the local Ae. aegypti population, so that it can rapidly recolonize the area. The limitations of each of these methods are illustrated in detail. It is recommended that vector control programs adopt an integrated vector management approach to conduct area-wide
control of *Ae. aegypti*. Among needed developments are: ovicides, longer-lasting larvicides, and effective, inexpensive vector surveillance tools.

**Acceptability and sustainability of intervention methods for *Aedes aegypti* control in urban and semirural areas**

Claudia M. Romero-Vivas and Andrew K. Falconar

*Universidad del Norte, Barranquilla, Atlántico, Colombia*

Presently, Colombia has the second highest incidence of dengue fever cases in the Americas, with control activities focused on the immature stages of *Aedes aegypti* in large water-storage containers. Biological (local fish, copepods) and chemical (temephos) control were used in 2 semirural and 2 urban areas, respectively, within the Department of Atlántico. A questionnaire related to water-storage containers and the acceptability/sustainability of the selected intervention methods was applied to 202 households. For rural and urban areas, the percentage of houses with a regular piped water supply was 51.6% and 93%, respectively, and the most frequent container types were tanks (71%) and plastic drums (42%), respectively. Although fish showed 100% efficacy and the copepods did not control *Ae. aegypti* larvae in the field, both methods were highly accepted by the rural community (100% and 86%, respectively); around 85% showed interest in long-term use of both biocontrol methods and would produce the local fish and copepods for their containers and for the community. In urban areas, only 35% and 33% of the community will welcome and accept the local use of fish and copepods, respectively; 84% welcomed the use of temephos. Overall, the community accepted education as an intervention method (93%) and 75% of those interviewed were willing to be part of a team that educated the community. In this study, biological control was accepted and may be sustainable in rural areas while in urban areas, temephos was appropriate; however, if insecticide resistance occurs, methods other than local fish and copepods need to be further evaluated because of their low acceptability.

**Entomological surveillance of *Aedes aegypti* in crowded human populations**

Juan F. Martínez-Perales,1 Argentina A. Garza-Robledo,1 Norma Lugo-Guillen,1 Carlos H. Marin-Hernandez,1 Violeta A. Rodríguez-Castro2 and Humberto Quiroz-Martínez2

1Servicios de Salud de Nuevo Leon, Monterrey, Nuevo Leon, Mexico; 2Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico

In recent years, a concern among health officials is about possible dengue transmission in areas with crowded human populations. To learn more about this, an entomological survey was carried out in elementary schools. Five ovitraps were placed in several schools, each one was examined weekly, and the school’s name, the strip number, and number of eggs were recorded, and traps were refilled with water and replaced. High densities of eggs were detected in some schools, and a high transmission risk was projected in those places, and insecticides were applied in the area.
Co-occurrence of *Aedes aegypti* and *Aedes mediovittatus* in aquatic habitats in southeastern Puerto Rico

Roberto Barrera, Manuel Amador, Andrew J. MacKay, Belkis Caban, Veronica Acevedo and Gilberto Felix

*Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico*

*Aedes mediovittatus*, the Caribbean tree hole mosquito, is a competent vector of dengue viruses, although it has not been incriminated as a vector in nature. It is not clear whether vector control programs should target this species or not. Previous studies using ovijars in the City of San Juan, Puerto Rico, revealed that *Ae. mediovittatus* was present in urban areas and areas of low-density housing. In this study, we investigated the distribution and co-occurrence of *Ae. mediovittatus* and *Ae. aegypti* pupae in water-filled containers in a rural municipality of Puerto Rico. Pupal surveys were conducted in 9 localities of the Municipality of Patillas (1,956 houses, 5,917 water containers). *Aedes aegypti* was more prevalent (House Index 21.1%) and abundant (6,207 pupae) than *Ae. mediovittatus* (10.1%, 2,603 pupae). Both species overlapped in 6% of the houses and 1.8% of the containers. Yet there were 2 localities where *Ae. mediovittatus* was more prevalent than *Ae. aegypti*. Both species occupied essentially the same type of artificial containers, although *Ae. mediovittatus* was not found in water tanks or in animal drinking pans with *Ae. aegypti*. Most *Ae. aegypti* were found in buckets, discarded containers, miscellaneous containers, plant pots, and barrels. Most *Ae. mediovittatus* were found in discarded containers, tires, buckets, barrels, and miscellaneous containers. These results indicate that the distribution of *Ae. mediovittatus* extensively overlaps with humans and *Ae. aegypti*.

**Selection of oviposition sites by female *Aedes aegypti***

Humberto Quiroz-Martinez,1 Mara I. Garza-Rodriguez,2 Martha I. Trujillo,3 Ingrid C. Compean-Ortiz,1 Juan F. Martinez-Perales2 and Violeta A. Rodriguez-Castro1

1Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, México; 2Servicios de Salud de Nuevo Leon, Monterrey, Nuevo Leon, México

Selection of oviposition sites by female *Aedes aegypti* and the consequences of this decision on the survival of offspring were examined. Traps with untreated water and water treated with spinosad and temephos were exposed to oviposition of *Ae. aegypti* females for 3 days. After that, the number of eggs laid was recorded and later all strips with eggs were returned to the container in order to produce mosquito larvae. Mosquitoes oviposited in all treatments. Traps with temephos had the highest risk of having mosquito larvae; because of that fewer eggs were laid, oviposition occurred mainly in traps with untreated water and spinosad; not a single larva survived where spinosad was applied.

**Electrophysiological and behavioral response of *n*-heneicosane, the oviposition pheromone of *Aedes aegypti*, on *Aedes albopictus***

Paula V. González, Paola A. González Audino and Héctor M. Masuh

*Pests and Insecticides Research Center, Villa Martelli, Buenos Aires, Argentina*

*Aedes aegypti* and *Ae. albopictus* are highly anthropophilic mosquito species and vectors of dengue viruses. The location of suitable sites for oviposition requires a set of visual, tactile, and olfactory cues that interact with the female before she lays her eggs. In this study, we evaluated the effect of *n*-heneicosane, a recognized oviposition pheromone of *Ae. aegypti*, on the olfactory receptors of the antennae of *Ae. aegypti* and *Ae. albopictus* using electroantennographic detection coupled with gas chromatography. We also analyzed the effect of this compound on the oviposition response of *Ae. albopictus* in order to determine whether the *n*-heneicosane also influences the behavior of this species. We observed a significant electroantennographic response to *n*-heneicosane in females of both species of mosquitoes. As this pheromone elicited electrophysiological response in *Ae. albopictus*, we also evaluated the oviposition behavior responses of this species, determining whether *n*-heneicosane could be used in bailed traps to improve monitoring and control strategies against both species of mosquitoes.

**Use of ovitraps to incriminate vertical transmission of *Aedes albopictus* as a vector of dengue viruses in two suburban localities of Santiago, northeastern Mexico***

Olga S. Sanchez-Rodriguez, Raul Torres-Zapata, Maricela Laguna-Aguilar, Marcela S. Alvarado-Moreno, Rosa M. Sanchez-Casas and Ildefonso Fernandez-Salas

*Facultad Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico*

In Nuevo Leon state in northeastern Mexico, dengue virus (DENV) is transmitted by *Aedes*
Aedes aegypti. However, it is documented that *Ae. albopictus* is also capable of transmitting DENV. In addition, vectorial competence of this species is well known and the virus infects female ovaries. Thus, vertical DENV transmission represents a risk to trigger dengue outbreaks under low mosquito population densities. This mosquito is a vector that has been found in some areas of Nuevo Leon state, including Santiago city, near Monterrey, Mexico. Santiago reports high numbers of dengue cases weekly during the dengue season. Strong suspicions of *Ae. albopictus* as an active DENV vector have been considered in this region. The aim of this study was to detect natural field infections of vertically transmitted DENV in *Ae. albopictus* in Santiago city. Ovitraps were used to collect *Ae. albopictus* in an effort to incriminate it as responsible for cases of DENV in Santiago. Two hundred ovitraps were placed in backyards and vacant lots: 100 in the Mahuacates study site and 100 in Panteon San Marco. A group of 177 were recovered and checked for *Aedes* eggs (89%) after 7 days. Of 177 ovitraps, 147 (83%) were positive for *Ae. albopictus* eggs. In Mahuacates, 6,199 eggs were collected and 2,370 were collected in Panteon San Marco. Mahuacates had 81% positivity and Panteon San Marco had 84% for *Ae. albopictus* eggs. Other mosquito species were also collected in Panteon San Marco, such as *Culex* spp. and *Ae. aegypti*, while in Mahuacates *Toxorhynchites rutilus* was found. Adult *Ae. albopictus* reared from eggs collected from ovitraps will be tested for DENV using reverse transcriptase–polymerase chain reaction to determine serotype, genotype, and phylogenetic relationships.

**Evaluation of antiadhesive materials for preventing the oviposition of Aedes aegypti**

Marcela S. Alvarado-Moreno, Maricela Laguna-Aguilar, Rosa M. Sanchez-Casas, Olga S. Sanchez-Rodriguez and Ildefonso Fernandez-Salas

Facultad Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico

*Aedes aegypti* is an important vector of dengue fever (DF). Every year >10 million DF cases are reported in the world. The mosquito's egg has a chemically resistant chorion that is invisible to conventional inspection. The chorionic pad keeps the egg moist and fixed in the breeding site. The egg is the most numerous stage of the life cycle. To date, *Ae. aegypti* eggs have generally been ignored as an object for control programs. This research was aimed to evaluate potential antiadhesive plastic materials acting over sticky exochorionic compound. The rationale was to induce the egg to sink and, therefore, prevent embryos in the eggs from hatching. Gravid females were used for these insectary trials. Three repetitions for each material were conducted and results recorded every 24 h. Preliminary data showed that only 3 materials had greater blocking activity: cellophane paper, polyvinyl chloride, and high-density polyethylene, 26.7%, 12.8%, and 5.2%, respectively. Field trials are underway for the 2011 mosquito season.

**Lethal ovitraps as a strategy for vector control of Aedes aegypti in Medellín, Colombia**

Marcela Quimbayo,¹ Gabriel Parra,² Carolina Torres,¹ Raul Rojo¹ and Guillermo L. Rua-UrIBE¹

¹Universidad de Antioquia, Medellín, Colombia; ²Universidad CES, Medellín, Colombia; ³Secretaria de Salud de Medellín, Medellín, Colombia

In Colombia, dengue is the main vector-borne disease due to the large number of cases and is present in several major urban centers of the country. The absence of a vaccine for disease prevention has meant that control measures are mainly focused on the reduction of the vector (adulticides, larvicides, and elimination of breeding sites); however, these methods are expensive. A less expensive alternative for vector control are lethal ovitraps (LO). In this study we evaluated, under laboratory conditions, different types of LO through a factorial design 33. The following insecticides were used: permethrin, temephos, and deltamethrin. Hay infusion (10% and 20%) and dechlorinated water were evaluated as oviposition attractants. The oviposition substrates that were compared included Dacron, towel, and flannel. We recorded the number of eggs per LO and monitored mortality of larvae, pupae, and adults. A statistically significant difference was observed between the types of insecticides tested, the attractive sources, and oviposition substrates. In the 2nd stage of this study, assessed under field conditions, the LO yielded better results than in the laboratory.

**A lethal ovitrap for Aedes aegypti control**

Emilia Seccacini,¹ Laura Juan,¹ Patricia Eisenberg,² Maria Cecilia Lorenzo,² Héctor M. Masuh,¹ Eduardo Zerba¹ and Susana Licastro¹

¹Pests and Insecticides Research Center, Villa Martelli, Buenos Aires, Argentina; ²Instituto Nacional de Tecnología Industrial (INTI), San Martín, Buenos Aires, Argentina

*Aedes aegypti* is the main vector of dengue fever in Latin America. It generally inhabits...
urban areas (indoors) and deposits its eggs in man-made containers. Ovitraps were developed as a surveillance tool and have been used for detecting and monitoring vector populations. Only in a few cases has it been used for vector control, incorporating an insecticide-impregnated strip. The present study evaluates the performance of newly designed ovitraps incorporating in the plastic matrix pyriproxyfen as a slow-release insecticide that prevents larvae from developing into adults. Polyethylene films impregnated with 0.1%, 0.5%, and 1% pyriproxyfen were added to vessels containing water and 3rd and 4th Ae. aegypti instars. A 100% adult emergence inhibition (EI) was obtained for all the concentrations studied. Films kept in water were active for at least 60 days. The same films used as ovitraps for gravid females produced 100% adult EI of the eggs laid on the water. An adult EI of 100% was obtained with ovitraps made of paraffin, estearine, or polyethylene containing 0.5% pyriproxyfen. This new ovitrap that continuously releases an insecticide into the water could be an additional tool for mosquito control, especially when ovitraps remain in the environment as new oviposition sites in dengue-endemic areas.

Evaluation of chlorine bleach formulations as ovicides for the control of Aedes aegypti

Andrew J. MacKay, Belkis Caban, Veronica Acevedo, Manuel Amador, Roberto Barrera and Gilberto Felix

Dengue Branch, Centers for Disease Control and Prevention, San Juan, Puerto Rico

We evaluated the potential of chlorine bleach formulations to eliminate eggs of Aedes aegypti in natural habitats. Aedes aegypti mosquitoes deposit their eggs in a variety of natural and artificial container habitats, typically in a band above the surface of the water. The eggs become resistant to desiccation after completing embryogenesis and can remain viable for many months. It was reported that a small proportion of eggs of a Puerto Rican strain of Ae. aegypti remained viable for >21 months. Dormant eggs may frustrate vector control efforts by providing a reservoir of mosquitoes that enables the vector population to quickly recover from short-term perturbations caused by control measures targeting the larval or adult stages. The purpose of this study was to identify minimal effective concentrations of different formulations of NaOCl that can be used as spray applications on representative substrates to prevent Ae. aegypti eggs present from hatching. We tested 3 formulations (Clorox® regular bleach, Clorox regular bleach formulated with 3% [w/w] washed smectite clay, and Clorox ProResults Outdoor bleach cleaner) on 3 types of substrates: polypropylene plastic, rubber, and concrete. The results indicated significant variation in the effectiveness of the formulations depending on the type of substrate.

Susceptibility to temephos and enzymatic activity in populations of Aedes aegypti from Venezuela during 2008 and 2010

Leslie Alvarez,1 Milagros Oviedo,1 Gustavo Ponce2 and Adriana E. Flores2

1Universidad de los Andes Venezuela, Trujillo, Trujillo, Venezuela; 2Universidad Autonoma de Nuevo Leon, San Nicolas, Nuevo Leon, Mexico

In 2008 and 2010, 2 populations of Aedes aegypti larvae collected from Lara and Trujillo states in Venezuela were tested using the World Health Organization bioassay technique to determine susceptibility to temephos. The activities of α- and β-esterases, mixed-function oxidases (MFO), glutathione S-transferase, and insensitive acetylcholinesterase (iAChE) were assayed in microplates. The New Orleans (NO) strain of Ae. aegypti was used as a reference and the threshold criteria for each enzyme were the highest NO absorbance values. The results showed susceptibility to temephos in the populations studied during both years, with resistance ratio LD50 values lower than 5. Elevated α- and β-esterases were present in the population for 2008 and for 2010 MFO and iAChE, while the population from Pampanito showed elevated α-esterases only altered enzymatic mechanism.

Temephos resistance in Caldas, Colombia: Current state and detection of biochemical mechanisms implicated in two autochthonous populations of Aedes aegypti

Lorena I. Orjuela,1 Manuela Herrera,1 Idalyd Fonseca2 and Martha L. Quinones1

1Public Health, Universidad Nacional de Colombia, Bogota, DC, Colombia; 2Universidad de Antioquia, Medellin, Colombia

The use of the organophosphate larvicide temephos is one of the main control measures used for dengue vector control in Colombia. The development of insecticide resistance is a threat to control efficacy. In this study, the level of susceptibility of Aedes aegypti to temephos was evaluated in 2 autochthonous populations in La Dorada, Caldas (Las Margaritas and Las Ferias neighborhoods), during 2007 and 2008. World Health Organization bioassays were carried out using the diagnostic dose of 0.02 mg/ml, and the
resistant ratio (RR) was calculated for each population. The results showed high levels of resistance to temephos in both populations, with RR higher than 10:11.48 in Las Margaritas and 13.27 in Las Ferias. Enzymatic biochemical assays were carried out to define the mechanisms involved in this resistance. Four enzymes were studied: nonspecific esterases, mixed-function oxidases, glutathione $S$-transferases, and insensitive acetylcholinesterases. Biochemical assays did not detect alteration profiles. More studies are required to establish if mechanisms like acetylcholinesterases could be implicated in temephos resistance in Caldas Ae. aegypti populations.

**Susceptibility to chlorpyrifos in pyrethroid-resistant strains of Aedes aegypti from the east coast of Mexico**

Adriana E. Flores, Nallely Banda, Brenda G. Silva, Humberto Quiroz-Martinez and Gustavo Ponce-Garcia

Laboratory of Medical Entomology, UANL, San Nicolas de los Garza, Nuevo Leon, Mexico

Chlorpyrifos resistance of 6 field strains of Aedes aegypti females from Veracruz, Mexico, was investigated and compared with a susceptible strain (New Orleans) with the bottle bioassay. The strains used were resistant to d-phenothrin, permethrin, deltamethrin, lambdacyhalothrin, bifenthrin, cypermethrin, alpha-cypermethrin, and $z$-cypermethrin. Results showed that strains from Tantoyuca, Veracruz, and Coatzacoalcos were susceptible to chlorpyrifos with resistance ratio (RR) $< 5$. The strains from Martinez de la Torre and Poza Rica were tolerant (RR $> 5 < 10$) and only the Cosoleacaque strain was resistant to chlorpyrifos. The practice of utilizing a single insecticide until the appearance of resistance has become standard practice that unfortunately rapidly reduces the number of insecticides available for effective vector control. The use of chlorpyrifos could have a tremendous potential for implementation in the Ae. aegypti control program in Mexico.

**Fitness costs of insecticide resistance in Aedes aegypti artificially resistant to lambdacyhalothrin**

Idalyd Fonseca-González,1 Duverney Chaverra Rodríguez2 and Nicolás Jaramillo-O.1

1Instituto de Biología, Universidad de Antioquia, Medellin, Colombia; 2Grupo Biología y Control de Enfermedades Infecciosas, Universidad de Antioquia, Medellin, Colombia

Insecticide resistance has become an obstacle for dengue control strategies worldwide. It has been proposed that there is a fitness cost associated with insecticide resistance. Understanding these potential costs is critical for developing efficient vector control strategies. This work evaluated the fitness costs under laboratory conditions of a strain of Aedes aegypti artificially selected for resistance to the pyrethroid lambda-cyhalothrin over 9 generations, in comparison with a control strain established from the same parental population and maintained under the same conditions and number of generations, but not exposed to selection for insecticide resistance, and the susceptible Rockefeller strain. For this purpose, horizontal life tables constructed from 5 cohorts of 100 mosquitoes were evaluated and compared between the resistant strain, a control strain, and the Rockefeller strain for the following parameters: developmental time to adult, adult daily survival rates, female longevity, adult fecundity, fertility, sex ratio, and net reproductive rate. The preliminary results showed that the resistant strain differs significantly from the control strain and the Rockefeller strain in the survival curves, female longevity, fecundity, and net reproductive rate ($P < 0.005$). We did not find significant differences in the rate of hatching, developmental time to adulthood, or the sex ratios among the 3 strains. The strain maintained without selection showed daily survival rates, fecundity, and net reproductive rates similar to the Rockefeller strain but statistically different from the selected strain. These results indicate that fitness costs occur in adults and could be associated with reproduction of the insecticide-resistant mosquitoes, suggesting a potential use in dengue vector control activities.

**Experimental trials of bed nets impregnated with Fendona® for control of Aedes aegypti**

Humberto Quiroz-Martinez,1 Violeta A. Rodriguez-Castro,1 Juan F. Martinez-Perales2 and Maria I. Garza-Rodriguez1

1Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico; 2Servicios de Salud de Nuevo Leon, Monterrey, Nuevo Leon, Mexico

Bed nets impregnated with insecticide are attracting increasing interest to control Aedes aegypti. This study was performed in order to measure the efficacy of bed nets impregnated with Fendona® for Ae. aegypti control. Bed nets impregnated with and without insecticide were placed over beds, and cones with 20 female Ae. aegypti were placed in a top, middle, and bottom section of the bed net; mortality was registered after 24 h of exposure. Impregnated bed nets showed high control efficacy of Ae. aegypti females.
Effectiveness of space spraying application of Aqua Reslin Super® and Aqua-K-Othrin® in the context of dengue control

Jose G. Ordoñez,1 Jaime Thirion,2 Arturo Losoya2 and Americo D. Rodriguez1

1Instituto Nacional de Salud Publica, Tapachula, Chiapas, Mexico; 2Buyer de México S.A. de C.V., Mexico, DF, Mexico

The spatial application of insecticides plays a significant role in vector control programs and in some situations is seen more as political decision than a strategic one. Nonetheless, it is necessary to assess the effectiveness of the insecticides applied as ultra-low volume sprays. In the Regional Centre of Investigation in Public Health in Tapachula, Mexico, we evaluated Aqua Reslin Super® (permethrin) and Aqua-K-Othrin® (lambdacyhalothrin) in lines and with obstacle tests. Insecticides were applied following the recommended instructions using an Aedes aegypti strain from Tapachula, Chiapas, Mexico. This strain was resistant to pyrethroids (43% mortality to permethrin and 51% to lambdacyhalothrin). In line tests, mortality registered in some replicates reached 100% at 100 m with Aqua Reslin. With Aqua K-Othrine, mortality reached 100% up to 40 m and 73.3% at 80 m. In tests with obstacles, mortality reached 100% in the front garden and 80% in the living room with both products, and in the backyard 86.7% and 93.3% with Aqua Reslin Super and Aqua K-Othrine, respectively. It is inferred that resistance in the Tapachula strain is mostly metabolic, and if knock-down resistance (KDR) is present, it is at very low frequency. Results suggest that these products still respond favorably to the dose of 10 and 1–2 g AI/ha, respectively, in areas where KDR resistance is not present.

Field evaluation of a new combined larvicidal–adulticidal ultra-low volume formulation against Aedes aegypti in Colombia

Martha L. Quinones and Natali Ortiz

Public Health, Universidad Nacional de Colombia, Bogotá, Colombia

Evaluation of alternatives to temephos for dengue vector control is necessary, due to the increasing presence of resistant Aedes aegypti populations in Colombia. A new ultra-low volume (ULV) formulation, containing permethrin as an adulticidal active ingredient and the insect growth regulator (IGR) pyriproxyfen as a larvicide, was evaluated in a field trial in San Joaquin, Cundinamarca. Weekly pre- and postintervention surveys were conducted in treated and control areas, evaluating the number of containers with larvae, larval density, and pupal index using the sweeping method, and adult density as the number of adult mosquitoes collected for 10 min in each house. Sentinel cages containing adult mosquitoes and jars containing Ae. aegypti larvae were placed outside during insecticide treatments. An 80% reduction in adult density was found, and lasted for 2 wk after treatment. Also, a 54% and 27% reduction in larvae and pupae density, respectively, was observed, lasting for 6 wk after treatment. A 95% inhibition in adult emergence was found in the sentinel jars. This new adulticide and IGR ULV formulation shows promise as an alternative to temephos.

An analysis of the species diversity and geographical distribution of sand fly (Diptera: Psychodidae) fauna from Quintana Roo, Mexico

Emigdio May-Uc,1 Hector A. Hernandez-Arana,1 Camila Gonzalez,2 Christopher Stephens3 and Eduardo A. Rebollar-Tellez3

1El Colegio de la Frontera Sur (ECOSUR), Chetumal, Quintana Roo, Mexico; 2Centro de Ciencias de la Complejidad C3, UNAM, Mexico, DF, Mexico; 3Universidad Autónoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico

A faunistic study was carried out to gain insight into the distribution, species diversity, and abundance of sand flies in Quintana Roo, Mexico. Even though Quintana Roo is one of the states in Mexico most affected by leishmaniasis, to date there have been no studies of the distribution of local sand fly species. Therefore, the objectives of this study were to determine the species diversity and abundance of sand flies in 3 regions of the state of Quintana Roo. From January to early April 2008, sand fly collections were made in 18 selected sites throughout Quintana Roo. Collection of flies was made using baited Disney traps, Centers for Disease Control and Prevention light traps, and Shannon traps. A total of 14,532 specimens were captured and comprised 2 genera and 20 species, two of which were new species. Overall, the northern region of Quintana Roo appeared to have the highest species diversity and abundance of sand flies in 3 regions of the state of Quintana Roo.
may be the main factors mediating the differences in the sand fly diversity and abundance in the various regions of Quintana Roo; however, at this time, we cannot conclude the spatial scale at which those environmental factors operate.

**Sand fly species (Diptera: Psychodidae) from diurnal resting sites and within a karstic cave in the Yucatan Peninsula, México**

Ana C. Montes-de-Oca-Aguilar,1 Alejandro D. Moo-Llanes,2 and Eduardo A. Rebollar-Tellez3

1Instituto Tecnológico de Conkal, Conkal, Yucatan, Mexico; 2Centro Regional de Investigación en Salud Pública, Tapachula, Chiapas, Mexico; 3Universidad Autónoma de Nuevo Leon, San Nicolas de los Garza, Nuevo León, Mexico

Phlebotomine sand flies are the vectors of Leishmania spp. parasites. Studies on the biology and ecology of this insect group have mainly been focused on the epidemiological aspects of parasite transmission. Despite the medical importance of sand flies, some basic biological and ecological aspects of these flies are still unknown. For example, “Where do sand flies hide during the daylight hours?” is one of the most frequent questions asked. Therefore, in the present study our objectives were to search for and determine adult resting sites for sand flies, as well as to document the daily activity of sand flies inhabiting a karstic cave. The search for resting sand flies was conducted from November 2007 to April 2008 in several locations of the Yucatan Peninsula. Resting flies were captured using a portable backpack aspirator. On the other hand, daily activity of sand flies inhabiting the karstic cave was carried out in November 2008 in a selected site in the state of Yucatan. Flies in the cave were caught using Centers for Disease Control and Prevention light traps. All flies were preserved in 8-ml plastic vials containing 70% ethanol. In total, we collected 72 sand flies in 18 out of 103 tree trunks (17.5%) that were aspirated, whereas in the karstic cave we collected a total of 703 flies. Within a karstic cave, 7 species were collected: Brumptomyia hamata, Lutzomyia beltrani, L. cayennensis maciast, L. cratifer, L. deleoni, L. serrana, and L. trinidadensis. There were apparent peaks of activity for the most common species from around 1600 to 2000 h and from 2000 to 0000 h.

**Susceptibility and enzymes associated with malathion and deltamethrin in four populations of Aedes aegypti from Venezuela**

Leslie Alvarez,1 Milagros Oviedo,1 Gustavo Ponce2 and Adriana E. Flores3

1Universidad de los Andes Venezuela, Trujillo, Venezuela; 2Universidad Autónoma de Nuevo Leon, San Nicolas, Nuevo Leon, Mexico

The LD₅₀ was determined for malathion and deltamethrin in populations of Aedes aegypti from Tres Esquinas, Pampanito, Úreña, and Lara, Venezuela, using the bottle bioassay, as well as resistance ratios (RR) compared to the insecticide-susceptible New Orleans strain. The populations were selected with each insecticide and quantified α- and β-esterases, mixed-function oxidases, insensitive acetylcholinesterase, and glutathione S-transferase (GST) using the microplate technique. Montella’s criterion was applied and ANOVA and Tukey test of significance (α = 0.05) were evaluated. The LD₅₀ to deltamethrin ranged from 0.027 to 0.053 μg/bottle. Mosquitoes from Lara, Tres Esquinas, and Pampanito showed loss of susceptibility at this insecticide (RR 9.05×, 8.6×, and 6.9×, respectively). The LD₅₀ for malathion ranged from 1.2 and 4.3 μg/bottle. The low RR values indicated loss of susceptibility in Tres Esquinas, Úreña, and Pampanito; on the other hand, Lara was resistant (RR 13.7×). β-esterases and α-esterases were observed as an altered enzymatic mechanism associated with malathion resistance found in Lara, as well as GST with the loss of susceptibility to deltamethrin.

**Breeding sites and seasonality of Anopheles species in the Puerto Carreño municipality, Vichada, Colombia**

Juan S. Durán,1 Jan E. Conn2 and Helena L. Brochero1

1Universidad Nacional de Colombia, Sede Bogotá, Bogotá, Colombia; 2Wadsworth Center, Albany, NY 12201

Puerto Carreño is endemic for malaria transmission because of appropriate geographical and ecological conditions combined with an influx of symptomatic individuals from surrounding rural areas. This study examined breeding sites, using species composition, diversity, and abundance during a 9-month period. Species identified were Anopheles marajoara s.l. (170), An. darlingi (68), An. braziliensis (67), An. oswaldoi s.l. (5), and An. argyritarsis (2). During the rainy season, the predominant species was An. marajoara s.l.; An. darlingi was more common during the dry season. We inspected 21 mostly permanent breeding sites: 12 (57%) excavations, 3 (14.3%) lagoons, 3 small riverbanks (14.3%), 2 (9.5%) flooded pastures, and 1 (4.8%) morichal palm pond. Anopheles marajoara s.l. (111/170) and An. braziliensis (60/67) were associated with exca-
vations characterized by direct sunlight and scarce vegetation. *Anopheles darlingi* (52/68) preferred small, shaded riverbanks with emergent vegetation and near dense vegetation patches. These excavations result from displaced human populations using soil for wall construction. During malaria outbreaks, local intervention consists of biological control using *Bacillus sphaericus*. To avoid the establishment of urban malaria under such conditions, we recommend 1) that control strategies be focused on surveillance for symptomatic persons, and 2) the creation of a binational program with Venezuela to offer diagnosis and adequate, immediate treatment.

**Biological aspects of Anopheles spp. in Puerto Carreño, Vichada, Colombia**

Irene P. Jiménez,1 Diana C. Suárez,1 Jan E. Conn2 and Helena L. Brochero1

1Facultad de Agronomía, Universidad Nacional de Colombia, Bogotá, Colombia; 2Griffin Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY 12201

Puerto Carreño, Colombia, shares a frontier with Venezuela. The forced displacement of residents from rural to urban areas in search of better opportunities due to internal armed conflict, coupled with the recognition of anophelines in the town, has resulted in an increasing number of malaria cases in this municipality. Mosquitoes were collected using human landing catchs indoors and outside dwellings between 1800 and 0600 h for 50 min/h for 2 consecutive nights/month for 8 months. We inspected urban breeding sites and analyzed malaria cases per month. The abundance of species collected was *Anopheles darlingi* (79.5%, n = 949) followed by *An. marajoara* s.l. (15.6%, n = 186), and *An. braziliensis* (5%, n = 59). *Anopheles darlingi* had 2 peaks of biting activity indoors (1900–2000 h; 2100–2200 h) and outdoors (1900–2000 h; 2100–2200 h), while *An. marajoara* s.l. had 1 peak indoors (1800–2100 h) and 2 peaks outdoors (1800–1900 h; 2000–2100 h). There was no correlation between adult and larval abundance for either *An. marajoara* or *An. darlingi*. These results suggest that most transmission occurs early in the evening when people are not protected by insecticide-treated nets. We recommend that malaria control strategies be focused on adequate diagnostics, immediate treatment for symptomatic people, and control of immature forms in urban breeding sites. Adult mosquito control efforts should be undertaken during epidemic situations to interrupt transmission.

**Parasitism by the nematode *Romanomermis iyengari* in breeding sites of *Anopheles pseudopunctipennis* in Oaxaca, Mexico**

Rafael Perez, Gonzalo Flores, Sabino Martinez and Jaime Ruiz

Instituto Politecnico Nacional, Oaxaca, Mexico

We evaluated the application of the parasitic nematode *Romanomermis iyengari* in 3 malaria-endemic communities in Oaxaca, Mexico. Previously, maps of the communities were made with all the breeding sites, and the pretreatment density of mosquito larvae (density of mosquito larvae before the treatments) was determined. The parasite applications were made in all the breeding sites, which varied in size from 80 to 8240 m². Three days later, data on percentage of parasitism and intensity of infection were collected and 7 days after the application data on posttreatment mosquito larvae density were collected. The application rate was 2,000–3,000 nematodes/m². Parasitism of mosquito larvae ranged from 71% to 100%, and the population reduction of the mosquito larvae ranged from 79% to 88%. In 60% of breeding sites, *R. iyengari* was recycled and persisted in the breeding sites, so the application of nematodes controlled larval *Anopheles pseudopunctipennis* populations.

**Cryopreservation of the mosquito parasite *Romanomermis iyengari***

Rafael Perez,1 Edward Platzer2 and Bradley Hyman3

1Instituto Politecnico Nacional (CIIIDIR OAXACA), Oaxaca, Oaxaca, Mexico; 2Department of Nematology, University of California, Riverside, Riverside, CA 92521; 3Department of Biology, University of California, Riverside, Riverside, CA 92521

The mermithid nematode *Romanomermis iyengari* is a parasite of mosquito larvae and has a high potential for reducing mosquito populations. It is important to develop new technologies for the preservation and distribution of infectious material useful for mosquito control. We developed a cryopreservation procedure for preservation of mermithid nematode eggs. Initially, we evaluated the use of different sucrose concentrations to prevent *R. iyengari* eggs from hatching during isolation from maintenance culture medium. The efficacy of different dimethyl sulfoxide (DMSO) concentrations to prevent ice crystal formation during the cryopreservation process was also evaluated. The cryopreservation procedure was optimized with 0.34 M sucrose and 2.5% DMSO. Samples of nematode eggs were stored in liquid nitrogen at −189°C and after
1–8 days of storage, egg samples were thawed and DMSO removed by washing. Egg hatching, nematode viability, and the infectivity of J2-stage nematodes to *Culex pipiens* and *Aedes aegypti* mosquito larvae were measured. Hatching occurred in most egg samples. In 4 cryopreserved preparations, preparasites emerging from frozen eggs successfully infected mosquito larvae. These results make available a new process for conservation of mermithid nematode species and provide a more reliable method for transporting infectious material to biocontrol sites.

**Diversity of aquatic insects associated with larval breeding sites of Culicidae of medical importance in eight states of Mexico**

J. G. Bond,1 Mauricio Casas-Martinez,1 Arnoldo Orozco-Bonilla,1 Humberto Quiroz-Martinez,2 Rodolfo Novelo-Gutiérrez,3 Armando Ulloa,1 Trevor Williams2 and Carlos F. Marina1

1Centro Regional de Investigación en Salud Pública Instituto Nacional Salud Pública, Tapachula, Chiapas, Mexico; 2Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de Los Garza, Nuevo León, Mexico; 3Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico

This study was conducted to determine the abundance and diversity of aquatic insects associated with mosquito breeding sites in 8 states of Mexico. An entomological survey was carried out in Guerrero, Oaxaca, Chiapas, Sinaloa, Nayarit, Michoacan, Jalisco, and Colima states, and all breeding sites were sampled and characterized. Aquatic insects were sampled with an entomological net and preserved in 96% ethanol, taken to the laboratory, and identified to genus and species using taxonomic keys. The diversity of aquatic insects was estimated using the Shannon index. The accuracy of index values was estimated by the Jackknife method and confidence intervals for the statistic were calculated by Bootstrap with replacement. A total of 321 collection sites were surveyed, and the total taxonomic diversity consisted of 11 orders, 64 families, 173 genera, and 246 species (4,688 individuals) of aquatic insects. The highest state diversity index was found in Chiapas (3.71) and Michoacan (3.61) did not differ significantly, followed by Sinaloa (3.54) that registered significantly lower aquatic insect diversity. Jackknifing indicated that diversity index values were slightly underestimated in all cases, but the magnitude of the error was small (1.18%). The most abundant culicid predators taken across all sampling sites were *Tropisternus* spp. (Coleoptera: Hydrophilidae) and the predatory bugs *Pelocoris* spp. (Hemiptera: Nauoridae) and *Ranatra* spp. (Hemiptera: Nepidae) that occurred at frequencies of 47.7%, 21.2%, and 16.0%, respectively.

**Efficacy of high-resolution satellite imagery to detect Anopheles albimanus habitat in Haiti**

Kevin A. Caillouet,1 Mark A. Rider2 and Michael V. Campbell3

1Center for Environmental Studies, Virginia Commonwealth University, Richmond, VA 23284; 2Tulane University, New Orleans, LA 70118; 3US Army Corps of Engineers, Alexandria, VA 22315

In the Artibonite Valley of Haiti, the peak in human malaria appears to be closely related to precipitation, lagging behind the peak of the rainy season by 2–3 months. Precipitation-mediated malaria suggests that spatial expansion of larval habitats and a high abundance of mosquitoes may be necessary for epidemic human malaria to occur in this region. During the height of the rainy season (July 21–29), standing water was surveyed across 3 elevation strata (valley floor, foothills, and mountains) in a 16-km2 scene centered on Deschappelles, Haiti. The spatial extent of all standing water within this small test area in the upper Artibonite Valley was then mapped using 40-cm-resolution Geoeye imagery, acquired on August 18, 2010. A hybrid supervised classification workflow was developed to delineate potential *Anopheles albimanus* habitats, including: wet agricultural lands, seasonal pools and wetlands, and drainage ditches. Building rooftops were also delineated to quantify host population distribution in Deschappelles. A quantitative accuracy assessment showed varying thematic accuracies among land cover classes, ranging from <50% (drainage ditches) to >80% (rice fields). Efficacy of the high-resolution imagery to detect these epidemiologically important surface features is discussed. The ability to accurately depict and quantify the spatial footprint of potential larval habitats is central to determining the relationship between larval habitat expansion and the transmission of malaria in Haiti.

**Malaria vectors involved in the urban transmission in Miraflores, Guaviare, Colombian Amazonia**

Laureano Mosquera,1 Irene P. Jiménez,2 Helena L. Brocher3 and Jan E. Conn1

1Unidad de Entomología, Laboratorio de Salud Pública–Secretaría Departamental de Salud del Guaviare, San José del Guaviare, Guaviare, Colombia; 2Facultad de Agronomía, Universidad Nacional de Colombia, Bogotá, Colombia; 3Griffin Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY 12201
Miraíflora, a municipality in the Department of Guaviare, Colombia, is located at 213 m above sea level and has rainforest characteristics and tropical wet forest. The urban area is located on the left bank of Vaupe River. Drastic changes in the ecosystem have cut and burned the forest for crops and livestock and have created risk factors that favor urban malaria transmission. Miraíflora has the highest annual parasite index (103 cases per 1,000 inhabitants) for malaria in Guaviare, with transmission of *Plasmodium vivax* (63 cases per 1,000 inhabitants), *P. falciparum* (39 cases per 1,000 inhabitants), and mixed malaria in an “endemic channel” (historic epidemiologic curve) depicting a permanent state of “outbreak.” In the urban area, anophelines were captured using human landing catches inside and outside dwellings between 1800 and 0600 h for 50 min/h for 2 consecutive nights in the dry and rainy seasons. The predominant species was *Anopheles darlingi* (80%), followed by *An. marajoara* sensu lato (15%) and *An. oswaldoi* (5%). *Anopheles darlingi* was caught throughout the night, with 2 peaks of biting activity: indoors (1900–2100 h and 0100–0200h) and outdoors (2000–2100 h and 0400–0600 h), showing an endophilic and endophagic behavior. The main anopheline oviposition sites were inspected and 15 urban breeding sites were characterized: excavations (*n* = 8), small river-banks (*n* = 6), and lagoon (*n* = 1). In the rainy season, the water levels of Vaupe River rose, flooding the municipality and forming one large breeding site for malaria-carrying mosquitoes. Based on epidemiological data, malaria case occurrence was detected every month for the last 5 years. An inverse relationship between the number of malaria cases and rainfall was found and the highest rate of transmission was recorded in the dry season. With epidemiological data, geographic maps, environmental, socioeconomic, and entomological characteristics, there was a focus on the risk of urban transmission and discriminated against in high, medium, and low as an important input for decision-making vector control. Therapeutic actions should be strengthened to ensure proper diagnosis and treatment for malaria through the training of primary health care personnel.

The length of the gonotrophic cycle of *Anopheles darlingi* under controlled laboratory conditions

Martha L. Ahumada,1 Patricia Gutierrez,1 Lorena I. Orjuela,1 Daniel Ruiz2 and Martha L. Quinones3

1Instituto Nacional de Salud, Bogota, DC, Colombia; 2Escuela de Ingeniería de Antioquia, Medellín, Colombia; 3Universidad Nacional de Colombia, Facultad de Medicina, Bogota, DC, Colombia

Increases in ambient temperatures have been associated with an increase in malaria transmission in Colombia. The purpose of this study was to evaluate the effects of temperature on the gonotrophic cycle length in *Anopheles darlingi*, the main malaria vector in Latin America. Mosquitoes were collected with the human landing captures and transported alive to the laboratory. After arrival, blood was provided and fully fed females were selected and kept in climate chambers at 24, 27, and 30°C. Bloodfed females were held in a container with water for oviposition and assessed at 12-h intervals. The mean duration of the gonotrophic cycle was 5.0 days (95% CI: 4.79–5.21), 4.36 days (4.22–4.49), and 3.64 days (3.44–3.83) at 24, 27, and 30°C, respectively. A 3°C rise in temperature produced a reduction of about 1 day in the length of the *An. darlingi* gonotrophic cycle. These results are being used in Colombia by the Integrated National Adaptation Program Project to fine-tune the parameters of dynamic malaria models in order to better estimate the malaria basic reproductive rate. According to these results, increases in temperature related to global changes in climatic conditions are likely to enhance the vectorial capacity of this primary malaria vector and, hence, lead to a further increase in the incidence of this disease in Colombia.

**Anthropophilic biting activity of Anopheles (Kerteszia) neivai associated with the activities of fishermen in a malaria-endemic area in the Colombian Pacific**

Jesús E. Escovar,1 Ranulfo González,2 Martha Quinones,1 Richard Wilkerson4 and Bruce Harrison5

1Universidad Nacional, Universidad de La Salle, Bogota, Colombia; 2Universidad del Valle, Cali, Valle del Cauca, Colombia; 3Universidad Nacional, Bogotá, Colombia; 4Smithsonian Institution–Walter Reed Biosystematics Unit, Washington, DC 20307; 5North Carolina Department of Environmental and Natural Resources, Winston-Salem, NC 27107

On the southwest Pacific coast of Colombia, a field study was initiated to determine the human-biting activity of *Anopheles (Kerteszia) neivai* in places frequented by fishermen, including nearby houses. Mosquitoes were collected over 24-h periods and during periods of biting activity in mangrove swamps, marshlands, fishing vessels in 3 locations, and outdoors and indoor houses. Of the total 4,745 mosquitoes collected, *An. neivai* accounted for 78.4% (3,721) and *An. albimanus* 21.6% (1,024). *Anopheles neivai* was most abundant in the mangroves and vessels (90.8%), while *An. albimanus* represented 82% of the indoor collections and 73% of the outdoor collections. In the mangroves
and fishing vessels, *An. neivai* showed biting activity throughout the day, with a peak between 1800 and 1900 h (52.7 mosquitoes per man-hour), and 2 minor peaks, one between 2100 and 2300 h (13.1 per man-hour), and another between 0500 and 0600 h (4.9 per man-hour). These peaks coincided with fishing activities in the marshlands and mangroves, a situation that puts the fishermen at risk of acquiring malaria while fishing. It is recommended that protective measures be implemented to reduce the risk of the fishermen getting malaria during their daily activities.

Detection of knockdown resistance (1014) mutation in *Culex quinquefasciatus* from Nuevo León, México

Gustavo Ponce,1 Karla Saavedra,2 Saul Lozano3 and Adriana E. Flores1

1UANL, San Nicolás de los Garza, Nuevo León, Mexico; 2Colorado State University, Fort Collins, CO 80523

The knockdown resistance (Kdr) target site to pyrethroids and DDT is linked to point mutations in the sequence of the *para*-type voltage-dependent sodium channel gene. This has been reported in many insect species and is characterized by a reduced sensibility of the insect’s nervous system to these compounds. *Culex quinquefasciatus* is an important vector of several encephalitides. We analyzed strains of *Cx. quinquefasciatus* from Nuevo León State, México. In this study, using polymerase chain reaction, a molecular tool based on test genomic DNA of each mosquito sampled, we found the Kdr mutation (leucine to phenylalanine at position 1014) was present in some samples. Currently, the major emphasis in resistance research is on the early detection of the molecular mechanisms of resistance for rational resistance management, with a view to controlling the development and spread of resistance in vector populations through better planning of insecticide usage.

Bloodfeeding arthropods from National Natural Park Laguna del Otún, Colombia

Ligia I. Moncada,1 Sebastián Mantilla,2 Nubia E. Matta3 and Maria C. Carrasquilla1

1Facultad de Medicina, Universidad Nacional, Bogotá, Cundinamarca, Colombia; 2Departamento de Biología, Facultad Ciencias, Universidad Nacional de Colombia, Bogotá, Cundinamarca, Colombia; 3Ministerio de la Protección Social de Colombia, Bogotá, Cundinamarca, Colombia

The Paramo ecosystem in Latin America ranges from Costa Rica to Chile. In Colombia, there are few studies about the bloodfeeding fauna inhabiting this ecosystem. This work reports the hematophagous fauna found in Laguna del Otún. The Centers for Disease Control and Prevention (CDC) light traps baited with CO2, CDC traps with an ultraviolet light, and an oil trap were used in the study. In some cases, in order to capture a swarm, the CDC inverted trap was used in addition to human landing collections. For immature stages of Simuliidae, streams were checked while standing water was checked for Culicidae. Ectoparasites were also collected from birds. A total of 125 adult insects were collected: 111 were identified as *Culicoides marinkelli*, 3 *Simulium rubiginosum*, 4 *Gigantodax misitu*, 4 *G. arreartorum*; and 24 *Aedes* spp. Larvae of immature black flies were identified as *G. misitu*, *G. arreartorum*, *S. stelliferum*, and *S. mariavulcanoae*. Two *Ixodes* spp. ticks were collected from birds. From this information, it is important to identify their hosts and determine their role in the transmission of vector-borne pathogens to the wild fauna.

Improvement of caged-mosquito field bioassays utilizing a modified wind-sensitive vane

Griffith S. Lizarraga

Clarke, Roselle, IL 60172

Caged-mosquito field bioassays are an important tool for corroboration of ultra-low volume adulticide efficacy. Such work is vital to the mosquito control and research communities. However, comparable demonstration research is also used, with slight variation, in agriculture and other fields related to public health. During 2009 and 2010, a review of field equipment and methodologies associated with caged-mosquito trials occurred, including incorporation of a previously published wind-sensitive vane used to autocorrect the position of bioassay cages. Several simple improvements enhanced the vane described by Vessey and facilitate the caged-mosquito field bioassay process. Data from demonstration research performed in both Arizona and Texas using Duet™ was presented to highlight the value of the improved “Vessey Vane.”

Infecitivity of nematode *Strelkovimermis spiculatus* in *Culex quinquefasciatus* larvae

Rafael Perez, Maricela Canseco, Jaime Ruiz and Sabino Martinez Tomas

Instituto Politecnico Nacional, Oaxaca, Mexico

We evaluated the effect of the parasitic nematode *Strelkovimermis spiculatus* against *Culex quinquefasciatus* larvae. To evaluate the
parasitic capacity of *S. spiculatus*, 5 doses of nematodes were applied (20, 15, 10, 5, and 3:1); the percentage of parasitism (PP) and intensity of infection (IINF = number of nematodes per mosquito larva) was determined. A PP of 100% was recorded for 5 doses and IINF of 11.15, 9.65, 7.05, 4.20, and 2.65 at doses 20, 15, 10, 5, and 3:1, respectively. To evaluate the effect of different doses of *S. spiculatus* nematodes in breeding sites of 1 m², 500 2nd instars were applied in 3 different doses (1,000, 3,000, and 5,000 nematodes/m²). A PP of 100%, 97.5%, and 80% and IINF of 5.4, 3.5, and 1.2 nematodes were registered with the doses of 5,000, 3,000, and 1,000 nematodes/m², respectively. To evaluate nematode recycling in 1-m² breeding sites, 500 2nd instars and 5,000 nematodes were applied (dose 10:1) for a period of 24 wk. We observed the recycling of the nematode, obtaining PP of 12.5% to 100% and median infection from 0.13 to 18.43 nematodes per mosquito larva.

Evaluation of a low-cost trap for the surveillance of mosquito vectors in Escobedo, Nuevo Leon, Mexico

Maricela Laguna-Aguilar, Olga S. Sanchez-Rodriguez, Marcela S. Alvarado-Moreno, Rosa M. Sanchez-Casas, Ildefonso Fernandez-Salas and Eduardo A. Rebollar-Tellez

FCB–UANL, San Nicolás, Nuevo León, Mexico

Vector surveillance of dengue viruses has been hampered by the lack of an effective trap that is inexpensive and noninvasive. Carbon dioxide microbial and affordable materials are options analyzed in this study. The aim of this investigation was to design a mosquito trap using affordable materials with a low-cost bait and with local production potential. Mosquito Adult Small-Trap (MAS-Trap) is a black plastic box with a funnel top, a collection bag, and a motor. The bait is a mixture of water, sugar, and baker’s yeast. In field trials conducted in northeastern Mexico, we carried out a randomized block design, using 3 treatments (trap with bait, trap without bait, and trap with sugar water) in 5 blocks. Three traps were placed outside the home for 24 h with the different treatments after which the number of mosquitoes that were caught was recorded. Differences were observed in trap collection efficiency between blocks (F = 9.8) and treatments (F = 3.5), but the addition of microbial bait did not increase the trap efficiency. The trap without an attractant had the highest collection mean. The analysis was performed using the statistical package SPSS with *P* = 0.05 effectiveness. Under these conditions, MAS-Trap has the potential to become an effective tool for collecting mosquitoes indoors and outdoors. It can be produced at a low cost by local vector control programs and can be operated with a minimum expenditure of energy. The MAS-Trap will now be tested in the field with different indices of infestation.

Frequency of the knockdown resistance ILE1,016 mutation in *Aedes aegypti* in Mexico

Quetzaly Siller,1 Gustavo Ponce,1 Adriana E. Flores1 and Aldo Ortega2

1Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico; 2Parasitología, Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, Mexico

The knockdown resistance ULE1,016 mutation was discovered in strains of *Aedes aegypti* resistant to permethrin on Isla de Mujeres, Quintana Roo, Mexico. Recent studies have reported the presence of this mutation in mosquitoes from different localities in Mexico, as well as an increase in the frequency of this mutation from 1997 to 2009. To determine the frequency of the Ile1,016 mutation as well as its genetic equilibrium according to the Hardy–Weinberg principle, 790 *Ae. aegypti* mosquitoes were sampled from 14 localities in Mexico from 2007 to 2009. The DNA was extracted from each mosquito using the salt technique, and polymerase chain reaction analysis was performed to amplify the wild-type allele (Val1,016) and the mutated allele (Ile1,016), which confers resistance to permethrin. A χ² test was carried out to determine the genetic equilibrium for the locus studied. We found that the Ile1,016 mutation was present in the 17 strains studied, 3 of which had high frequencies: ACA9 (0.97), IGU9 (0.9265), and SAN9 (0.90). Strains PAN7, VER7, COS9, COA9, TAN9, ACA9 (0.97), IGU9 (0.9265), and SAN9 (0.90) all showed a genetic disequilibrium. The presence of high frequencies of the Ile1,016 mutation in *Ae. aegypti* is due mainly to the selection pressure of pyrethroid insecticides, particularly permethrin.

Potential vectors of *Dirofilaria immitis* in dogs from inland Yucatan, Mexico

Pablo Manrique-Saide,1 Daly Martínez-Ortíz,1 Cedric Castillo-Cohuo,1 Manuel E. Bolío-González,2 Jorge C. Rodríguez-Buenfil,3 Javier Escobedo-Ortega,4 Roger I. Rodriguez-Vivas,5 Silvia Hernández-Betancourt1 and Juan Chablé-Santos1

1Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 2Departamento de Medicina Interna y Cirugía, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 3Departamento de Genética Animal, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 4Departamento de Medicina Interna y Cirugía, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 5Departamento de Medicina Interna y Cirugía, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico;
Mosquito collections were made on microfilaremic dogs, positive for *Dirofilaria immitis*, for 10 consecutive nights in Molas (Municipality of Merida, Yucatan, Mexico) during the rainy season (August) in 2010. A total of 496 mosquitoes (385 females and 111 males) of 6 species were collected: *Aedes aegypti*, *Culex quinquefasciatus*, *Cx. interrogator*, *Cx. coronator*, *Ae. taeniorhynchus*, and *Psorophora ferox*. *Culex quinquefasciatus*, *Ae. taeniorhynchus*, and *Ae. aegypti* were the species most frequently collected, and they had the highest rate of feeding success (the percentage of the total catch for each species that was bloodfed) and the highest attack rates (the number of mosquitoes trapped in dogs per hour of mosquito exposure). Filarial worms (L1 and/or L3 instars of *D. immitis*) were microscopically observed in all mosquito species collected except *Ps. ferox*, with a filarial infection rate of 9.6% of total examined mosquitoes. Further confirmative polymerase chain reaction analysis is currently underway. These preliminary results are part of a larger body of work undertaken in this area to understand the epidemiology of *D. immitis* in the Yucatan Peninsula.

A targeted intervention to reduce rain-filled buckets/pots against two nontargeted interventions for *Aedes aegypti* control in Merida, Mexico

Pablo Manrique-Saide,1 Eduardo A. Rebollar-Téllez,2 Alejandra González-Moreno,3 Adán Zapata-Peniche,4 Guillermo Guillermo-May,1 Laura Buenfil-Silva1 and Mario Barrera Pérez2

1Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatan, Mexico; 2Departamento de Zoología de Invertebrados, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico; 3Departamento de Enfermedades Infecciosas y Transmitidas por Vector, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, Mérida, Yucatan, Mexico

We report results of a cluster-randomized, controlled trial evaluating the effect of targeting *Aedes aegypti* pupal production sites in Merida, Mexico. The methodology integrated 3 phases: 1) baseline pupal survey to identify the most productive containers; 2) interventions in 3 groups of Basic Geo-statistical Areas: one where buckets/pots were reduced–managed versus total elimination of rubbish from the backyards and traditional control carried out by health authorities; and 3) follow-up survey 2 wk after the intervention. Key outcome variables consisted in the number of wet containers, total positive containers (both for immature and pupae only), their relative contribution to total pupal production and pupae per person, and Breteau (BI) and house indices (HI). The baseline survey confirmed that buckets/pots were the most productive containers, producing 55.5% of pupae. After a targeted intervention based on the efficient use of abandoned buckets/pots, there was a substantial decrease (67%) in the number of immature and pupae-positive buckets/pots in the group with the bucket/pot management. The HI decreased (−6.9%); the PUHI [(number of houses infested by *Ae. aegypti* pupae/houses inspected) × 100] decreased (−31.3%); the BI decreased (−34.3%); and the pupae per person decreased (−96.6%) in the bucket/pot management group. *Aedes aegypti* pupae per container recorded in both bucket/pot management and tidy backyard groups decreased by ca. 30%, against a 75% increase in the control group.

Container types and characteristics associated with *Aedes aegypti* pupal productivity in localities of high risk of dengue transmission of Morelos State, Mexico

Pablo Manrique-Saide,1 Azael Che-Mendoza,2 Mariana I. González-Fernández,2 Cassandra González-Acosta,3 Alejandro Villegas-Trejo,3 Felipe Dzul-Manzanilla,2 William Cruz-Canto1 and Guillermo Guillermo-May1

1Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatan, Mexico; 2Departamento de Prevención y Control de Enfermedades Transmitidas por Vector, Secretaría de Salud de Guerrero, Chilpancingo, Guerrero, Mexico; 3Coordinación de Enfermedades Transmitidas por Vector y Zoonosis, Servicios de Salud de Morelos, Cuernavaca, Morelos, Mexico

Cross-sectional *Aedes aegypti* pupal surveys were performed by direct inspection inside and around 1,713 houses from 88 clusters (neighborhoods) during the dry and rainy seasons of 2008 in Cuautla, Jojutla, and Tlaquiltenango, Morelos State, Mexico. Every type of container was described and categorized according to their location (outdoors or indoors), material, and use (disposable or useful). All pupae were collected from each positive container. The
relative importance for each type of container considered their contribution (%) to total pupae production. Overall, 73–97% of containers with *Ae. aegypti* pupae were considered “useful” by the communities. During the dry season, large cement washbasins and potted plants produced 48% and 60–98% of total pupae collected in Cuautla, Jojutla, and Tlaquiltenango. During the wet season, the diversity of productive containers varied and increased: small diverse containers (21%) and buckets (19%) for Cuautla, potted plants (20%) and small diverse items (20%) for Jojutla, and small diverse (34%) and large cement washbasins (12%) for Tlaquiltenango. A targeted strategy focused on these containers could greatly improve current control efficacy and reduce *Ae. aegypti* populations. Future *Ae. aegypti* control program in Morelos will need to involve community participation and try to find communication/educational approaches through messages focused on the management of these containers.

**Identification of floral volatiles and their effect on *Aedes aegypti* and *Aedes albopictus***

Santiago Von Oppen, Héctor M. Masuh, Susana Licastro and Paola A. González Audino

*Pests and Insecticides Research Center, Villa Martelli, Buenos Aires, Argentina*

The use of nectar as an energy source for mosquitoes has been widely established. Although the selection mechanism of floral hosts is not fully understood, it has been proven that visual and odor cues are involved. In the search for new compounds that could be used as an additional tool for the attraction of mosquitoes to a trap, our laboratory began the study of volatiles obtained from different plant species (e.g., *Calendula* spp., *Primula* spp., *Lobularia* spp., *Plectranthus* spp., and *Bellis* spp.). In the present study, the floral volatile components were determined in optimized conditions, through the adsorption in solid-phase micro extraction and later analysis by gas chromatography–mass spectroscopy. In each species, the chemical components were separated and the major components were identified. The volatile source was either fresh plant material or a mixture of its pure constituents. In order to determine the effects of the flower volatile components on *Aedes aegypti* and *Ae. albopictus* mosquitoes, preliminary behavior assays of main compounds were performed in a Y-tube olfactometer. The aim of this study was to develop a cost-effective, environmentally friendly method of controlling the disease-transmitting *Aedes* mosquitoes.

**Effect of sublethal exposure of pyriproxyfen released in a fumigant formulation on *Aedes aegypti* fertility and fecundity**

Laura Harburguer, Eduardo Zerba, Héctor M. Masuh and Susana Licastro

*Pests and Insecticides Research Center, Villa Martelli, Buenos Aires, Argentina*

Insect growth regulators (IGRs) are considered a new generation of insecticides having great possibilities for insect control. The activity of IGRs generally results in the reduction of adult emergence of the target insect. However, they seem to have side effects, particularly on female reproduction following larval IGR treatments: for example, effects on fecundity (increase or diminution of the number of eggs laid) and on fertility (reduction of hatchability or viability of eggs). This study evaluated the effect of treatment with a sublethal dose of pyriproxyfen, released in a fumigant formulation, on fecundity and fertility of *Aedes aegypti*. Early 4th instars were treated with a dose that caused between 40% and 50% inhibition of adult emergence. Three to 4 days after emergence, surviving females were fed and mean number of eggs laid was noted. Ten to 14 days later, eggs were placed in water to evaluate their viability. Ovaries of surviving females were dissected and mean number of ovarioles was recorded. Results showed a decrease in the mean number of eggs laid by treated females with a reduction in viability of those eggs; however, no difference was observed in the mean number of ovarioles between treated and control females. This indicates that the use of this new formulation combined with an adulticide, as has been proposed in previous work from our laboratory, not only produce larval and adult mortality of those individuals present at time of application, but also cause a reduction in fertility and fecundity of surviving females.

**Droplet size and effectiveness on *Aedes aegypti* of an ultra-low volume adulticide–larvicide formulation using different solvents**

Laura Harburguer, Alejandro Lucia, Emilia Seccacini, Susana Licastro, Eduardo Zerba and Héctor M. Masuh

*Pests and Insecticides Research Center, Villa Martelli, Buenos Aires, Argentina*

One of the most common methods for controlling arthropod vectors, particularly mosquitoes, is the application of insecticides by ground sprayers. When selecting spray equipment and insecticides, factors such as the recommended dose, the droplet size, the mov-
ing speed of the vehicle, the sprayer cost, etc., are very important. Droplet size is one of the most significant factors that affects how well a vector control application works, and depends on, among other factors, the solvent used. The use of “gasoil” as a solvent is often recommended, invoking better insecticidal effect, more aerosol dispersion, and high visual impact at the time of application. In this work, we measured the droplet size during application of an ultra-low volume formulation containing permethrin as an adulticide and pyriproxyfen as a larvicide. This formulation was applied with a thermal fogger using water, diesel, or biodiesel as solvents, and with a water-based cold fogger. We evaluated the effectiveness of these formulations against late 3rd or early 4th instars and adults of *Aedes aegypti* under semi-field and field trials performed in Puerto Libertad, Misiones, Argentina. We found that the average droplet size of aqueous formulations was higher than in oil-based formulations. All formulations were equally effective on adults at all distances tested; however, the effect on larval mortality, measured as emergence inhibition (% EI), was higher for the aqueous formulation.

**Efficacy of chemical control interventions on *Aedes aegypti* in Guerrero, Mexico**

Felipe Dzul-Manzanilla,1 Pablo Manrique-Saide,2 Azael Che-Mendoza,1 Jose Herrera-Cardoso,2 Guillermo Guillermo-May,2 Audrey Lenhart,3 Philip J. McCall,3 Patricia Penilla4 and Americo D. Rodriguez4

1Departamento de Prevención y Control de Enfermedades Transmitidas por Vector, Secretaría de Salud de Guerrero, Chilpancingo, Guerrero, Mexico; 2Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 3Vector Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom; 4Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Publica, Tapachula, Chiapas, Mexico

The Mexican national dengue program employs for chemical interventions of *Aedes aegypti* control: 1) “Abatización” (1% granules of temephos, T.M.FOS®) for permanent breeding sites; 2) ground vehicle–mounted, ultra-low volume (ULV) space spraying (sumithrin, ANVIL® 2+2 ULV) in areas/clusters with dengue outbreaks and/or entomological risk; and 3) indoor rapid residual spraying (RRS) at houses of probable dengue cases (with a variety of insecticides). We report a comparison of the efficacy of interventions (single/combined and evaluated 24 h and 1 wk postapplication) on the prevalence/abundance of: 1) *Ae. aegypti* pupae in containers; 2) indoor-resting adults collected with backpack aspirators; and 3) gravid females inferred by ovitraps located outdoors in Iguala, Guerrero, Mexico. “Abatización” alone reduced pupal populations immediately by 100% and for 1 wk later. The ULV applications had a significant, immediate effect on oviposition rates (~30% and ~50% reduction on the prevalence of positive ovitraps and average of eggs/ovitrap, respectively) and was sustained for 1 wk. The RRS using lambdacyhalothrin (ICON® 10 PH) had an effect on the prevalence of houses with indoor-resting adults and their abundance after 24 h and for 1 wk later. The combined application of interventions reduced both pupae and adults for 24 h and 1 wk later. Results are discussed in the context for improving *Ae. aegypti* control in Mexico.

**Susceptibility of seven *Aedes aegypti* populations from Medellín, Colombia, to pyrethroid and organophosphate insecticides**

Jorge M. Cadavid, Marta Londono, Shirley Milán, Hillary Yepez and Guillermo L. Rua-UrIBE

Universidad de Antioquia, Medellín, Colombia

In Medellín, Colombia, *Aedes aegypti* is the main vector for dengue viruses. Besides the use of commercial insecticides in the community, authorities have used the organophosphate insecticide malathion to control dengue vectors since 1987. Between 2007 and 2008, 223,010 houses were fumigated indoors and 465 h of outdoor spraying was conducted with a truck-mounted machine. In 2003, studies showed that in the Buenos Aires neighborhood of Medellín, the population of *Ae. aegypti* was resistant to fenitrothion and, at the same time, the population from Santa Cruz neighborhood showed a resistance risk to the same insecticide, malathion, and to the pyrethroid deltamethrin. In this paper, we assessed the susceptibility of 7 *Ae. aegypti* populations from Medellin to pyrethroid and organophosphate insecticides. The results showed different susceptibility profiles, and the resistance seems to be a risk factor for dengue in the city. This paper contributes to the design of strategies for improved dengue vector control by Colombian authorities, based on field evaluations.

**Application of a geographical information system in establishing the dengue entomological risk in Medellín, Colombia**

Johana E. Restrepo,1 Gabriel Parra,2 Raul Rojo2 and Guillermo L. Rua-UrIBE3
Dengue is a serious public health problem in Colombia. Epidemiologic assessments of this disease have been made with geographical information systems (GIS), and it has been proven that this is a useful tool for the identification of risk areas for the dengue control. In this investigation, entomologic risk maps were created with GIS and different variables such as house, container, Breteau, and adult indices were analyzed and associated with the number of dengue cases and environmental and socioeconomic variables. The analysis of these patterns revealed that, for the city, the entomologic factors did not show a relationship with the number of cases during the years of our study, but a relationship between the environmental and socioeconomic variables was found. This study, through the establishment of risk areas, contributes to the design of correct and appropriate strategies for the prevention and control of this disease by the health officials in Medellin.

Genital morphology of male Trichoprosopon spp. males for the description of a new species of the pallidiventer complex

Juan D. Suaza,1 Sandra Uribe,1 Guillermo L. Rua-Urbe,1 Gonzalo Abril2 and Charles Porter1

1Universidad de Antioquia, Medellin, Colombia; 2Universidad Nacional de Colombia, Medellin, Colombia; 3Centers for Disease Control and Prevention, Atlanta, GA 30333

The genus Trichoprosopon is represented by 13 species in the Neotropical region. Some of these species are of medical importance because they are vectors for emerging and reemerging viruses. Some species of Trichoprosopon are known from Colombia, and there may be other undescribed species. With the objective of establishing new Trichoprosopon species (in the pallidiventer complex), larvae and pupae were collected from Guadua angustifolia stumps in the town of Jardin, Antioquia, and a series of adult specimens was obtained as well as the morphological description of the male genitalia. A detailed study of the diagnostic morphological structures was made at the Centers for Disease Control and Prevention in Atlanta, GA (USA), and at the National University of Colombia in Medellin, Antioquia. The structures were described using conventional microscopy and a scanning electron microscope. The morphological variations in the mesosome and in the dististyle were established, and these detailed characteristics were very useful for the assignment of the new species of this taxon. This new record contributes to our improved morphological and taxonomic knowledge of the country’s mosquito fauna and specifically their potential role as vectors within the pallidiventer complex.

Susceptibility status of Anopheles albimanus to insecticides used in public health in Atlántico, Colombia, in 2010

Paula X. Pareja Loaiza,1 Gabriela Rey Vega1 and Ronald Y. Maestre Serrano2

1Entomology, Instituto Nacional de Salud, Bogotá, Cundinamarca, Colombia; 2Universidad de Cartagena, Cartagena, Bolívar, Colombia

The department of Atlántico in Colombia is not in the endemic region for malaria; however, there are risk factors that have precipitated outbreaks of this disease during recent years. Anopheles albimanus is the main vector with a widespread distribution throughout the department. The insecticide susceptibility for this species is unknown and unavailable for the definition of prevention and control strategies. Its susceptibility to organochlorines, organophosphates, pyrethroids, and carbamates was evaluated in a population of An. albimanus from Santo Tomas, Atlántico, during 2010. Bottle bioassays were performed following the Centers for Disease Control and Prevention methodology, using doses and diagnostic time for deltamethrin (12.5 μg/30 min), lambda-cyhalothrin (12.5 μg/30 min), cyfluthrin (12.5 μg/30 min), permethrin (12.5 μg/30 min), alphacypermethrin (6.25 μg/30 min), alphanpermethrin (12.5 μg/min), DDT (100 μg/45 min), malathion (50 μg/30 min), fenitrothion (50 μg/45 min), and bendiocarb (12.5 μg/30 min) on a field population of An. albimanus mosquitoes. Three repetitions, each one with 4 replicates and 1 control, were carried out. The population of An. albimanus evaluated was susceptible to all insecticides evaluated. This result was probably due to the low selection pressure for insecticides because of the epidemiological profile of the disease in this zone. Therefore, we recommend the use of these insecticides to control future outbreaks of malaria in the region.

Laboratory evaluation of spatial repellency and irritability of permethrin for Culex quinquefasciatus

Rocio Ramirez-Jimenez,1 Ewry A. Zarate-Nahon2 and Ildesonfo Fernandez-Salas2

1Laboratorio de Entomologia Medica, Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico; 2Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico
West Nile virus (WNV; family Flaviviridae) infects >150 species of birds as well as mammals such as squirrels, dogs, wolves, horses, and mountain goats. Birds are the natural reservoir hosts, and WNV is maintained in nature in a mosquito–bird–mosquito transmission cycle primarily involving *Culex quinquefasciatus* (especially *C. bidens*, and *Angel Bricen Haemagogus* to several doses (0.025 nmol/cm²). *Uranotaenia* and *Cx. chloropterus* were the species with the broadest range in the eastern areas, WNV has been detected, but no vector control is focused on this virus because dengue is more important vector-borne disease. Therefore, this study examined the behavioral response of *Cx. quinquefasciatus* using the High-Throughput Screening System; this system has a modular design that examines several aspects (contact irritancy, spatial repellency, and toxicity). The behavior response of *Cx. quinquefasciatus* to several doses (0.025 nmol/cm², 0.25 nmol/cm², 2.5 nmol/cm², and 25 nmol/cm²) of topically applied permethrin was evaluated. At a concentration of 25 nmol/cm², 48.0% contact irritation was obtained; at a concentration of 25 nmol/cm², 4% toxicity was obtained; and at a concentration of 2.5 nmol/cm², a spatial repellency of 11.0% was obtained.

**An update on the mosquito fauna and its distribution in the Mexican state of Guerrero**

Felipe Dzul-Manzanilla,1 Azael Che-Mendoza,1 Pablo Manrique-Saide,2 Eduardo A. Rebollar-Téllez,3 Heron Huerta4 and Angel Briceño-Uc2

1Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 2Servicios de Salud del estado de Guerrero, Chilpancingo, Guerrero, Mexico; 3Laboratorio de Entomología Médica, Departamento de Zoología de Invertebrados, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico; 4Laboratorio de Entomología, InDRE, Mexico, DF, Mexico

The aim of this study was to review and update the species of mosquitoes and their distribution in the state of Guerrero, Mexico. We created a database containing historical and recent records from mosquito collections made by the Ministry of Health of Guerrero. This database was then exported to Maxent software (including bioclimatic variables) to create predicted distribution maps. We listed a total of 70 species belonging to 17 genera (in bold) in Guerrero: *Anopheles albimanus, apicimacula,* argyritarsis, crucians, eiseni, pseudopunctipennis, punctimacula; *Aedeomyia squamipennis; Aedes aegypti,* angustivittatus, atropalpus, epactius, guerrerio, idamus, lornarrean, nigromaculis, scapularis, serratus, taeniorynchus, terrenstortilis, trivittatus; *Haemagogus* anastasio-nis, equinus, mesodontentus; *Psorophora ciliata,* cilipes, confinnis, cyaneascens, ferox, howardi, luzii, totonaci, varipes; *Lutzia bigoti; Culex bideni,* cedecei, conspirator, corniger, coronator, erraticus, inflectus, interrogator, iolandmb, nigripalpus, pec-cator, pilosus, pinarocampa, quinquefasciatus, rest-uans, sandrae, stigmatsoma, tarsalis, thriambus, trifidus, schicki; *Deinocerites* howardi, pseudes; *Coquillettidia nigricans; Mansonia* inhabitans, tii-tllans; *Orthopodomyia kummi; Limatus durhamii; Sabethes* chloropterus; *Uranotaenia* lowii, pulcher-rima, saphirina; and *Toxorhynchites* (Tx.) grand-iouss. We report 3 new state records: *Tx. theobaldi,* *Caliseta* particeps, and *Wyeomyia ar-throstigma. Aedes aegypti,* *Cx. coronator,* and *Cx. quinquefasciatus* were the species with the broadest recorded and predicted distributions.

**An update on the scorpion fauna of Guerrero, Mexico**

Humberto Quiroz-Martinez, Irma G. Zepeda-Cavazos, Ilse A. Siller-Aguillon, Ingrid C. Compean-Ortiz and Violeta A. Rodríguez-Castro

*Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico*

More than 150,000 cases of scorpion sting intoxication, by far the highest rates in the world, are reported annually in Mexico. Considering that correct identification of scorpions is important to prevent and control their populations, we are currently updating the species of scorpions and their distribution in the Mexican state of Guerrero. Specimens were obtained from entomological surveillance collections carried out by Ministry of Health personnel and community-based monitoring, and from historical records and the National Commission for Knowledge and Use of Biodiversity (i.e., CONABIO) databases. We report 31 species in 5 genera (in bold) from Guerrero: *Bioculus parvulus; Centruroides* balsasensis, elegans, fulvipes, gracilis, lindipus, margaritatus septentrionalis, meisi, nigrescens, nigrimanus, nigrovittatus; *Diplolcentrus* bellar, coylei, majahuensis, magnus, tehuacanus; *Hoffmannihadrurus* gertschi; and *Vaejovis* acapulco, atenango, crassimanus, curvidigitus, mexicanus mexicanus, mexicanus smithi, noteno, occidentalis, punctatus spadix, punctatus variegatus, puritanus, pusillus, sprusei, vaquero. Three widely distributed *Centruroides* species (*C. balsasensis, C. meisi,* and *C. lindipus*) are important for public health and associated with the high number of human intoxication cases caused by scorpion stings.
Experimental trial of bed nets impregnated with Fendona® for control of Musca domestica (Diptera: Muscidae)

Irma G. Zepeida-Cavazos, Ilse A. Siller-Aguillon, Ingrid C. Compean-Ortiz, Violeta A. Rodriguez-Castro and Humberto Quiroz-Martinez
Universidad Autónoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico

Bed nets impregnated with insecticide are attracting increasing interest to control Musca domestica. Our study measured the efficacy of bed nets impregnated with Fendona® to control M. domestica. Bed nets impregnated with and without insecticide were placed over a bed. Twenty adult M. domestica were placed in World Health Organization cones on the top, middle, and bottom sections of the bed net. After 24 h of exposure, mortality was recorded. Insecticide-impregnated bed nets showed high control of adult M. domestica.

Morphological deformities in larvae of Chironomus spp. from Pesqueria River in Nuevo Leon State, Mexico

Jorge Pascual Martínez Muñoz, Gloria Isabel Flores Martínez, Laura Xochitl Mateos Lopez and Josefina Cecilia Trujillo García
Laboratorio Estatal de Salud Pública de Oaxaca, Oaxaca, Mexico

Morphological alterations of mouthparts in the larvae of midges have been related to water contamination by heavy metals. The objective of this study was to determine the frequency of deformities in larvae of Chironomus spp. in 2 localities of the Rio Pesqueria. The larvae were collected with a benthic net, preserved in ethyl alcohol, and identified and processed in the laboratory. Preparations for microscopic examination of the head were made, recording the type and frequency of deformities. The mentum was the most common site of morphological alteration, followed by the pecten, mandibles, and, finally, the antennae. No statistical difference was found between the localities sampled.

Triatominae (Hemiptera: Reduviidae) of Oaxaca, Mexico

Azael Che-Mendoza,1 Felipe Dzul-Manzanilla,1 Pablo Manrique-Saide,2 Jose Juan Molina,1 Heron Huerta,4 Vianney Vidal-Acosta4 and Alejandro Villegas-Trej5
1Departamento de Previención y Control de Enfermedades Transmitidas por Vector, Secretaría de Salud de Guerrero, Chilpancingo, Guerrero, Mexico; 2Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico; 3Laboratorio Estatal de Salud Pública, Galo Soberén, Secretaría de Salud de Guerrero, Acapulco, Guerrero, Mexico; 4Laboratorio de Entomología, InDRE, Mexico, DF, Mexico; 5Coordinación de Enfermedades Transmitidas por Vector y Zoonosis, Servicios de Salud de Morelos, Cuernavaca, Morelos, Mexico

Three of the most important genera (Triatoma, Rhodnius, and Panstrongylus) involved in the transmission of Chagas disease are present in Oaxaca state, Mexico. From July 1997 to July 2010, 7,191 triatomines were collected and identified from 157 municipalities. A total of 2,623 were adults, belonging to 3 genera and 9 species. In addition, there were 4,568 nymphs from different instars. The identified genera and species were Triatoma barberi, T. nitida, T. phyllosoma, T. mazzottii, T. pallidipennis, T. dimidiata, T. gestaeckeri, Rhodnius prolixus, and Panstrongylus rufotuberculatus. In total, 673 (25.6%) specimens had natural Trypanosoma cruzi infections. Rhodnius prolixus, T. phyllosoma, T. mazzottii, T. dimidiata, T. gestaeckeri, T. pallidipennis, T. barberi, and T. nitida presented high indices of natural Trypanosoma cruzi infection, while 206 (4.5%) nymphs were positive for Trypanosoma cruzi. A total of 7,023 triatomine bugs, 1,668 adults and 5,355 nymphs, were negative for natural Trypanosoma cruzi infections. The species with the highest density of Trypanosoma cruzi was T. dimidiata, whereas T. pallidipennis, R. prolixus, and P. rufotuberculatus had lower infection levels.

New distributional records of phlebotomine (Diptera: Psychodidae) sand flies in northern Mexico

Jorge J. Rodriguez Rojas,1 Ildefonso Fernandez-Salas,1 Angel Rodriguez-Moreno,2 Ingeborg D. Becker,3 Victor Sánchez-Cordero,4 Christopher Stephens4 and Eduardo A. Rebollar-Tellez1
1Universidad Autónoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico; 2Departamento de Medicina Experimental, Facultad de Medicina (UNAM), Mexico, DF, Mexico; 3Departamento de Medicina Experimental, Instituto de Biología (UNAM), Mexico, DF, Mexico; 4Departamento de Medicina, Instituto de Ciencias Nucleares (UNAM) & C3, Mexico, DF, Mexico

Cutaneous leishmaniasis is a tropical vector-borne disease and in México the highest incidence rates are reported in the southern states. Nevertheless, in northern Mexico, there have been reports of human cases in Coahuila (COA), Nuevo León (NL), and Tamaulipas (TAM). These Mexican states are located along the
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