ABSTRACT. The 19th Annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 75th Annual Meeting in New Orleans, LA, in April 2009. The principal objective, as for the previous 18 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 37 presentations that were given orally in Spanish or presented as posters by participants from 5 countries in Latin America, Puerto Rico, the United Kingdom, and USA. Topics addressed in the symposium included: surveillance, chemical and biological control, insecticide resistance and repellency of 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 Triatoma and Leishmania.

KEY WORDS Mosquito bionomics, control, insecticide resistance, dengue, malaria, Chagas disease, leishmaniasis

INTRODUCTION

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

This publication includes summaries of 37 presentations that were given in Spanish by participants from 5 countries in Latin America, Puerto Rico, the United Kingdom, and USA. Topics addressed in the symposium included surveillance, chemical and biological control, insecticide resistance and repellency of Aedes aegypti L.; distribution, behavior, 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 and Leishmania. Summaries of 16 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; and Clark and Rubio-Palis 2006, 2007, 2008).

SUMMARIES

Aedes aegypti dynamics in northern Puerto Rico

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Although dengue infections increase during the wettest part of the year in northern Puerto Rico, dengue viruses persist in an endemic state on the island throughout the year. One hypothesis explaining dengue endemism is continuous rainfall, i.e., rainfall occurs during every month of the year, producing enough Aedes aegypti mosquitoes to sustain virus transmission. A longitudinal study of Ae. aegypti adults and weather parameters was initiated in 2 urban areas in the City of San Juan in November 2007. Forty BG-Sentinel traps were placed at the entrance of houses in each area and were simultaneously operated for 4 consecutive days every 3rd wk. Although rainfall occurred during every month of the year, there were short periods (2–3 wk) without rainfall. There was a significant, positive effect of accumulated rainfall on adult Ae. aegypti productivity. On the other hand, mosquitoes did not completely disappear at times when there was no rainfall. Pupal surveys revealed the presence of containers holding water (buckets, rooting plants in water, leaking water meters, and some barrels) and producing Ae. aegypti in the absence of significant rainfall. Thus, dengue endemism is facilitated by mosquitoes produced in containers with water supplied by people.

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Breeding sites classification of *Aedes aegypti* in Monterrey, Nuevo León, Mexico

Juan F. Martínez-Perales,1 Carlos H. Marín-Hernández,2 Nemecio López-Vargas1, Gabriela Govea-López1, María T. González-Mendoza1, Humberto Quiroz-Martínez2 and Violeta A. Rodríguez-Castro3

Entomological surveys were conducted to detect adult *Aedes aegypti* production in the urban area of Monterrey, Mexico. The larval habitat site classification system recommended by the Secretary of Health was used to identify the most productive sites. Results showed highest production in breeding sites that were classified as “controllable,” followed by “destructible” breeding sites, and finally those where temephos had been applied. The most common breeding sites of *Ae. aegypti* occurred under accidental conditions in a house.

Effect of a clean yard on the *Aedes aegypti* larval index

Juan F. Martínez-Perales,1 Norma A. Lugo-Guillen1, Argentina A. Garza-Robledo1, Carlos H. Marín-Hernández2, Nemecio López-Vargas1, Gabriela Govea-López1, María T. González-Mendoza1, Humberto Quiroz-Martínez2 and Violeta A. Rodríguez-Castro3

A field test was carried out to study population behavior around the “clean yard” (“Patio Limpio”) program. In that program, the community’s knowledge about practicing cleanup activities related to the *Aedes aegypti* life cycle was evaluated before a community conference about *Ae. aegypti* and its control was presented. After the conference, the same community was evaluated. As a result, the larval index decreased from 12.0% in 2007 to 6.7% in 2008.

Efficacy of a smoke-generating tablet containing permethrin and pyriproxyfen against *Aedes aegypti*

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A new smoke-generating tablet containing pyriproxyfen (2%) and permethrin (10%) was evaluated in the laboratory and the field. Recovery of the insecticides from the fumes was over 90% for pyriproxyfen and around 50% for permethrin. The biological efficacy of pyriproxyfen released in the smoke was evaluated on 3rd and 4th instars using different doses and exposure times. Complete (100%) adult emergence inhibition (% EI) was obtained at 30 min, and a dose-dependent effect was observed at 5 min. The efficacy of the permethrin released was evaluated as a knockdown effect (T = time) (KT50) on adults. There was no significant difference in KT50 values obtained for permethrin and permethrin plus pyriproxyfen (KT50 = 19.87 and 19.36 min, respectively). A field trial was performed in Misiones, Argentina. In one area, 2–4 tablets were lit per house. ULV application of 15% permethrin from a handheld sprayer was performed in another area of the city (a positive control). A 3rd area was used as an untreated control area. Sentinel cages containing adults and jars with water and 3rd and 4th instars were used to determine treatment efficacy. Adult emergence inhibition (% EI) and adult mortality at 24 h were determined. Adult collections were conducted inside houses with a manual aspirator before and after treatments. The results showed more than 90% EI and almost 100% adult mortality in the area treated with the tablets. Based on adult *Ae. aegypti* house collections, 6 wk of significant control was achieved (P < 0.01).

Field evaluation of *Bacillus thuringiensis* var. *israelensis* DT and WDG and pyriproxyfen for the control of *Aedes aegypti* in Colombia

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Because of the increasing occurrence of temephos-resistant *Aedes aegypti* populations in Colombia, evaluation of alternative larvicides is necessary for the dengue control program. Although *Bacillus thuringiensis* var. *israelensis* (Bti) has been used in the country, a new formulation in tablets would facilitate community participation. Two formulations of Bti, DT and WDG (VectoBac™ and the IGR pyriproxyfen (Sumilavr™), were evaluated and compared with the use of temephos. Ten groups of 9 blocks were selected in 2 neighborhoods in the municipality of La Dorada, state of Caldas. Pre- and postintervention surveys were conducted in the middle block (of the 9 blocks) and evaluated. These surveys measured the number of positive containers, larval density, pupal index using the sweeping method, and adult density (i.e., the number of
The ability to enable an active compound to be delivered at a low rate while providing extended-release properties poses unique challenges. The results obtained with Natular™ DT (spinosad) offer the international market a truly revolutionary formulation to fight mosquito vectors of dengue viruses. This presentation discussed how this formulation was developed.

**Relative efficacy and persistence of synthetic deltamethrin-impregnated fabrics against Aedes aegypti under laboratory and field conditions**

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Insecticide-treated mosquito nets and curtains have recently received attention as means of mosquito control. This study was undertaken to evaluate deltamethrin efficacy and persistence on synthetic fabric curtains (Permanet™). We determined the effect of sunlight and dust on the insecticide persistence as well as the effect of washings, both in the field as well as under laboratory conditions. Performance was measured using standard World Health Organization (WHO) procedures: 3-min exposure, and “median time to knockdown” bioassay test with an insecticide-susceptible strain (Rockefeller) of *Aedes aegypti*. The results indicated that sunlight and dirt did not diminish the persistence of deltamethrin in field conditions after 6 months of exposure. Under laboratory conditions, mortality diminished, but no more than 50% after 60 days of exposure; however, this effect increased mortality considerably after 10 washings. Finally, it is recommended that these factors be considered when the use of insecticide-impregnated fabrics is included in the mosquito control program.

**Spatial distribution of entomological risk of transmission and dengue incidence in Medellin, Colombia**

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Medellin is an important city in Colombia where dengue is widely distributed. Nevertheless, such distribution is heterogeneous, making the establishment of massive control strategies difficult. In order to give priority to suitable strategies for vector control, it is necessary to define the spatial distribution of the disease and the entomological factors determining dengue risk. In the present study, the entomological risk of transmission of dengue in the city was evaluated following the World Health Organization’s parameters of risk. Periodic mosquito collections were conducted in 16 districts and 2 municipalities. The house, container, and Breteau indices were calculated. Using these indices, the incidence of the disease by district was determined. The cluster analysis of entomological factors the dengue incidence showed 5 great clusters in the city. One of these grouped 11% of the districts including 33.8% of all dengue cases. Results of this study indicated that the zones of greater entomological risk and higher dengue transmission information were relevant for the development of suitable control strategies.

**Use of a Dengue Decision Support System to manage geographic data for dengue cases in the city of Merida, Mexico**

Darwin Elizondo-Quiroga,1 Francisco Gamboa-Garcia,1 Saul Lozano-Fuentes,2 Jose A. Farfan-Ale,1 Maria A. Loroño-Pino,1 Julian Garcia-Rejon,1 Salvador Gomez-Carro,3 Victor Lira-Zumbardo,3 Rosario Najera-Vazquez,1 Pedro Gonzalez-Martinez,4 Chester G. Moore,5 Barry J. Beaty, and Lars Eisen2

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To improve management and control of arthropodborne diseases, we need to make better operational use of emerging technologies such as mapping software, geographical information systems, and the internet. In a collaborative effort between the Universidad Autónoma de Yucatán and Colorado State University, we are developing a Dengue Decision Support System (DDSS) to aid in the process of gathering and analyzing information, gaining new insights, generating alterna-
tives, and making decisions. We are field testing the DDSS together with Servicios de Salud de Yucatán in the city of Merida, Mexico. This requires management of geographic information, clinical data, and vector data. We provide an example of the way in which the DDSS can be used to manage and display laboratory-confirmed dengue cases. Using the geographic explorer included with the DDSS and global positioning system (GPS) receivers, we determined the locations (home addresses) of cases that occurred in Merida from 1997 to 2007. These locations and the date of onset of symptoms were used to generate a time lapse for Google Earth. The DDSS and its geographic explorer thus have the potential to provide better capacity for displaying space-time patterns of dengue outbreaks.

Leishmania mexicana infection among wild-caught sand fly species (Diptera: Phlebotomidae), of villages from Calakmul, Campeche, Mexico

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In Mexico, the main parasite causing cutaneous leishmaniasis (CL) is Leishmania mexicana, and the only proven sand fly vector is Lutzomyia olmeca olmeca. Nonetheless, evidence gathered from recent studies has found that other species may also act as vectors. To identify the medically important species, we conducted field studies using Shannon and Disney traps in 4 villages of Calakmul, an area that has had cases of CL. Collections were made in 2 transmission seasons from 2005 to 2007 in Unión 20 de Junio, Dos Lagunas Sur, Arroyo Negro, and Once de Mayo. Biting and infection rates were calculated for the most abundant species in each locality (i.e., Dos Lagunas Sur—Lutzomyia panamensis and Lu. olmeca olmeca; Unión 20 de Junio—Lutzomyia cruciata and Lu. olmeca olmeca; Once de Mayo—Lutzomyia shannoni, Lu. cruciata, and Lu. olmeca olmeca), while in Arroyo Negro, Lu. cruciata and Lu. olmeca olmeca exhibited the highest biting rate, respectively. We report the establishment of a polymerase chain reaction (PCR) protocol and validation of IR1 and LM17 oligonucleotides. The infection rates among the different field collection sites are compared and discussed.

Abundance and nightly activity behavior of a sylvan population of Triatoma dimidiata from the Yucatan, Mexico

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Triatoma dimidiata (Hemiptera: Reduviidae) is the vector of Trypanosoma cruzi in the Yucatan in Mexico. Earlier studies have shown that domestic and peridomestic populations of the vector originated from sylvan stock and that insecticide spraying could be affected by reinfections of houses by the sylvan population. In addition, most of the previous reports have focused on the domestic populations, and little is known about the sylvan population. The aims of our study were to determine the nightly activity patterns of adult T. dimidiata, and to document their reproductive status and T. cruzi infection rate. During 8 sampling nights from March to July, 2007, we collected 544 bugs in a selected location in the Yucatan. Illuminated traps were effective in attracting the sylvan individuals, and adults exhibited a bimodal activity pattern. The accumulated mean of captured bugs showed a nonlinear distribution: $Y = 0.90 + 17.88 \ln(X)$ for females and $Y = 2.44 + 11.64 \ln(X)$ for males. Furthermore, we found that both sexes were correlated with the mean temperature and mean relative humidity recorded during the sampling period. Of 46 dissected females, we found that 43.5% had fully formed eggs, and that 4.4% had sperm within the spermathecae. The infection rate of adults with T. cruzi was 3.7%.

Mosquito records from Mexico: 4. The mosquitoes of Guerrero State (coastal region)

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A study to determine the mosquito species that inhabit the coastal region of Guerrero State, Mexico, was conducted during the summer of 2008. The incidence of some diseases transmitted by mosquitoes has been high in recent years in Guerrero State; therefore, this study provides new information regarding mosquito distribution in the coastal region of the state, which could be useful in the study and control of these disease vectors. Immature and adult mosquitoes were collected in the field and transported to the State Health Laboratory of Guerrero and the Parasitology Laboratory of Autonomous Agrarian University “Antonio Narro” Laguna Unity, for mounting and identification. We obtained 12 genera, 15 subgenera, and 36 mosquito species, of which 6 are new records from Guerrero State.

Molecular systematics of the Oswaldoi Complex (Anopheles: Nyssorhynchus) in South America

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Anopheles (Nyssorhynchus) oswaldoi is known to consist of a complex of closely related species, due primarily to differences in reported malarial vector capacity and host preferences across its extensive range in Latin America. The species is reported from Panama in the north through to the north of Argentina. In this study, a detailed morphological and molecular investigation of specimens from the type locality in Espirito Santo, Brazil, ascertained the true identity of An. oswaldoi sensu stricto. Using the molecular identity of the nominotypical member of the complex as a foundation, deoxyribonucleic acid (DNA) sequences of the nuclear ITS2 and mitochondrial COI genes revealed 3 additional taxa (An. oswaldoi A, B, & C) from other areas in Brazil (An. oswaldoi s.s., A & B), Colombia (An. oswaldoi A, B, & C), Ecuador (An. oswaldoi B & C), Peru (An. oswaldoi C), Trinidad (An. oswaldoi B), and Venezuela (An. oswaldoi B). Morphological comparisons of the majority of samples of An. oswaldoi s.s. A & B showed consistent differences in the morphology of the male aedeagus. However, analysis of the male genitalia of a single isofamily of An. oswaldoi A from Mato Grosso, Brazil, showed polymorphism in the aedeagus, prompting reevaluation of male genitalia as a robust tool for differentiation of the sibling species in the Oswaldoi Complex.

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Phylogenetic analysis from Albitarsis Complex members using white gene

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Phylogenetic analysis of 5 species belonging to the Anopheles (Nyssorhynchus) albitarsis complex using mitochondrial COI and ND4 genes and nuclear rDNA ITS2 and 28S sequences resulted in conflicting tree topologies. A better result was obtained using white gene for 4 of the above species with intron retention noted in one (Anopheles marajoara). The discovery of a species related to An. marajoara (An. albitarsis F) in Puerto Carreño, Vichada, and Putumayo, Colombia, led us to repeat the white gene analysis with An. albitarsis F included. Alignment was unambiguous and done manually. The white gene sequences were analyzed using maximum parsimony (PAUP version 4.0b10) and Bayesian analysis (Mr. Bayes version 3.1). The An. marajoara intron, also absent from species F, was not included in the analyses. We sequenced 692 exon bases, of which 67 were parsimony informative: 8 at position 1, 5 at position 2, and 54 at position 3. Tree topologies were equal using both analytical methods, and Colombian and Brazilian An. marajoara clustered together in the basal clade. In all cases, the trees showed relationships as follows: outgroup(marajoara(albitarsis B(albitarsis F((deaneorum)(albitarsis s.s.))))). All species were monophyletic, and higher branch support was obtained using Bayesian analysis.

Reassessment of Anopheles in Colombia: Species verification using ITS2 and COI DNA sequences

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An aqueous extract was shown to inhibit the larvae of Anopheles oswaldoi and Anopheles albimanus species in Colombia. Larval mortality for Cx. quinquefasciatus and An. albimanus was determined with probit analysis. The LC50 for Cx. quinquefasciatus larvae was between 88% and 95%, whereas the range for An. albimanus was between 98% and 100%. The LC50 for An. albimanus (Barranquilla colony) larvae averaged 141 mg/liter, whereas larvae from the Cartagena colony averaged 103 mg/liter average. The R. graveolens aqueous extract was shown to be effective for controlling larvae of the 2 mosquito species that were analyzed.

**Effects of an aqueous extract of Azadirachta indica on the growth of larvae and development of pupae of Culex quinquefasciatus**

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The neem tree, Azadirachta indica (Meliaceae), is one of the most studied plant species for controlling pests, including mosquitoes. However, the effect of aqueous neem seed extracts (ANSE) on all 4 larval instars of Culex quinquefasciatus is unknown. To determine the effect of ANSE on the growth of the 4 larval instars and the pupal stage of Cx. quinquefasciatus, 8 bioassays using ANSE concentrations ranging from 30 to 1,000 ppm were applied to 1st-, 2nd-, 3rd-, and 4th-stage larvae. Mortality was assessed when 93% to 98% of the larvae reached the pupal stage in the untreated control. The growth of larvae and development of pupae were recorded to determine a growth inhibition index (GII), a relative growth inhibition index (GII), and the effective concentration (EC50) of ANSE for each larval stage. In the surviving population, the duration of larval stages and pupal viability were recorded. The EC of 460, 440, 410, and 550 ppm inhibited the growth of 50% of the population of the 1st, 2nd, 3rd, and 4th instars, respectively. Concentrations of ≥500 and 700 ppm applied to 1st and 2nd instars, respectively, prevented the development of pupae, and concentrations ≥700 ppm applied to the 4th instars prevented the emergence of adults.

**Land use, diversity, and abundance of immature anophelines in larval habitats in the Lower Caura River Basin, Bolivar State, Venezuela**

Jorge E. Moreno, Yasmin Rubio-Palis, Maria-pia Bevilacqua, Domingo Medina, Víctor Sán...
Malaria is a major public health problem in Venezuela, especially in the southern states, despite efforts in prevention and control. During 2007, Bolivar State reported 72% of the 41,000 malaria cases that occurred in the country. For the Caura River Basin’s indigenous populations, malaria is the most important health problem. In this pristine region, malaria is related to traditional agriculture and human population increase. In order to explain the relationship between malaria and deforestation, entomological evaluations of larval habitats were carried out. Anopheline larval surveys were conducted during March–November 2008 in 3 riverine villages: Surapire and El Palmar, inhabited by Amerindians of the Ye’kwana and Sanan ethnic groups, respectively, and Jabillal, inhabited by “criollos.” Simultaneously, environmental data and anopheline larval habitats were recorded. Larvae belonging to 8 Anopheles species were identified. The most abundant species was Chagasia bathana, followed (in order of abundance) by An. oswaldoi, An. triannulatus, An. mediopunctatus, and An. nuneztovari; the least abundant species was Anopheles darlingi. The larval density and species diversity were higher in the streams followed by flooding areas. Likewise, larval density was higher in El Palmar, while diversity was higher in Jabillal. The results suggest that anopheline abundance and diversity can be strongly related with landscape, deforestation, and exposure to sunlight. This project was funded by IDRC-CDLAC-18.

Abundance and biting activity of anopheles in the Lower Caura River Basin, Bolivar State, Venezuela

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Malaria is the most important vectorborne disease in Bolivar State, southern Venezuela, accounting for over 70% of the total number of malaria cases reported in the country. The Caura River Basin is a vast territory where malaria is endemic among indigenous populations, but there is no available information regarding vector species and its bionomics. A longitudinal study was conducted in 3 riverine selected villages: Surapire, inhabited by Amerindians of the Ye’kwana ethnic group; El Palmar, inhabited by the Sanan ethnic group; and Jabillal inhabited by “criollos.” Every 2 mo, mosquitoes were collected from sunset to sunrise during 3 consecutive nights in each village using CDC light traps and Mosquito Magnets™. To determine the biting activity of anophelines, Mosquito Magnet™ nets were changed every hour, and samples were kept moist inside polyporous boxes until they were killed and then identified the following morning. Mosquitoes were dried over silica gel for future identification of sporozoites by enzyme-linked immunosorbent assay (ELISA). Collections with CDC light traps yielded 6 species, the most abundant being Anopheles braziliensis and An. darlingi. Using the Mosquito Magnet™, up to 9 species were collected, the most abundant being An. darlingi and An. nuneztovari. In general, Jabillal had higher mosquito densities, accounting for 88.4% of total mosquitoes collected with the Mosquito Magnet™. Anopheles darlingi and An. nuneztovari showed similar biting patterns, with an early peak at sunset, although they were active throughout the night and showed a minor peak at sunrise. The CDC light traps collected large numbers of An. braziliensis in El Palmar but were ineffective in collecting An. darlingi and An. nuneztovari, the suspected vectors in this area. This project was funded by IDRC-CDLAC-18.

Determination of Anopheles species in Putumayo, southern Colombia, and their role in malaria transmission

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The department (State) of Putumayo, southern Colombia, is one of the main malaria transmission areas in Colombia. However, the role of the different species of Anopheles found in this region as malaria vectors is still unclear. In a previous study, the species An. rangeli and An. oswaldoi B were found naturally infected with Plasmodium vivax (Pv210). However, the most abundant species found biting humans was An. benarrochi...
B, which is considered one of the main vectors in the neighboring state of Loreto in Peru. This study aimed to clarify the role of this species in malaria transmission in southern Colombia. Mosquito samples were collected in 2007 and 2008 and preliminarily identified using morphological characteristics of the females. Some specimens were sequenced for ITS2 and COI. The separation between An. benarrochi B and An. oswaldoi s.l. was done by a PCR-RFLP using Hae III. All of the mosquitoes were analyzed by ELISA for P. vivax 210 and 247 and Plasmodium falciparum infections. In total, 4,315 specimens were tested, and 6 were found positive: 5 An. benarrochi B and 1 An. oswaldoi s.l. These results confirm the role of An. benarrochi B in malaria transmission in Putumayo, and its role as a new malaria vector in Colombia.

Abundance, composition, and biting behavior of anopheline mosquito species in Meta, Colombia

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To confirm the Anopheles species involved in the transmission of malaria, we characterized their biting behavior to support implementation of control strategies in the Department (State) of Meta, Colombia. Between June 2007 and July 2008, mosquito collections were made in 5 municipalities of Meta. For taxonomic identification, we used available entomological series. To establish the biting behavior, human landing catches were conducted indoors and outdoors. Twelve species of Anopheles were identified from their larval habitats: An. darlingi was the most abundant species (38.3%), followed by An. marajoara (26%), and An. rangeli (20%). We found An. nimbatus in Puerto Concordia. Of the 1,375 mosquitoes obtained with human landing collections, we found: An. darlingi (96.1%), An. braziliensis (1.6%), An. marajoara (1.5%), An. costai (0.3%), An. rangeli (0.2%), and An. peryassui (0.1%). Anopheles darlingi is present throughout the night, and there is variation in the biting activity and patterns of activity indoors and outdoors. In the municipalities where there were several species of Anopheles, An. darlingi was the main vector of malaria and was most abundant in the larval habitats and in human landing catches. Bed nets with insecticide can be one measure for malaria control because of the indoor activity of An. darlingi throughout the entire night.

Characterization of the Anopheles species present in 2 ecologically and epidemiologically different regions in La Guajira, northern Colombia

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Malaria transmission in Riohacha, on the coast of La Guajira, northern Colombia, is characterized by outbreaks, while 50 km away, inland and next to the Sierra Nevada de Santa Marta in Dibulla, malaria is endemic. A survey was conducted to determine the species composition, biting activity of the species present, and natural malaria infectivity in the 2 areas. Mosquito specimens were collected in 2007 and 2008 and were preliminarily identified using morphological characteristics of females and their progeny. For confirmation, some specimens were sequenced for ITS2 and COI. All mosquitoes were analyzed by ELISA for Plasmodium vivax 210 and 247 and P. falciparum infectivity. The species found in Riohacha were Anopheles aquasalis (417) and An. albimanus (67) biting humans, while in Dibulla, we collected An. darlingi (558), An. albimanus (168), An. triannulatus s.l. (30), An. pseudopunctipennis s.l. (10), and An. punctimacula s.l. (3). Out of 1,186 specimens analyzed by ELISA, only 2 An. darlingi from Dibulla were found positive for P. vivax (Pv210). The characteristics of the landscape and the presence of An. darlingi in Dibulla may explain the differences in malaria transmission between the 2 areas.

Differential effects of Bacillus sphaericus strain 2362 on Anopheles albimanus and Culex quinquefasciatus larvae in experimental conditions

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The objective of this study was to evaluate the toxicity of a Bacillus sphaericus granulated product to Culex quinquefasciatus (Sibaté and Villavicencio) and Anopheles albimanus (Cartagena and Barranquilla) larvae. The following concentrations of B. sphaericus were used: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, and 500 ppm for An. albimanus larvae, and 2, 4, 6, 8, 10, 12, 14, and 16 ppm for C. quinquefasciatus larvae. The exposure time was 48 h with 20 larvae and 3 repetitions per concentration. The laboratory temperature was 28°C ± 2°C. The LC50 and LC95 were determined by probit analysis. Concentrations between 8 and 12 ppm of B. sphaericus showed a
significant effect on larval mortality (80% and 100%) for Cx. quinquefasciatus, while for concentrations between 40 and 200 ppm, mortality was less than 50% for An. albimanus larvae. The probit LC50 was 192 ppm for An. albimanus larvae, whereas probit LC50 was 2.2 ppm for Cx. quinquefasciatus larvae. In conclusion, Cx. quinquefasciatus larvae were susceptible to B. sphaericus at lower concentrations (2 and 12 ppm), while An. albimanus larvae showed high mortality in 500 ppm of B. sphaericus.

Evaluation of the persistence of a commercial formulation of Bacillus sphaericus in anopheline larval habitats in Bolivar State, Venezuela

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Sifontes municipality in Bolivar State is the main malaria endemic area in Venezuela. During the last 5 years, an annual average of 14,000 cases has been reported, reaching 13,508 during 2007. The classic vector control measures, based on insecticide spraying, have been ineffective; hence, there is a need to implement new control strategies. A commercial formulation of Bacillus sphaericus (VectoLex CG™ 7.5%) was evaluated in larval habitats of Anopheles marajoara, An. triannulatus, and An. braziliensis. Two experiments were carried out in a lagoon and an inundated pasture area, where 2 and 3 g/m² doses were evaluated during 60 and 90 days. The results demonstrate differences in B. sphaericus persistence depending on the type of larval habitat and anopheline species present. The general trend indicates reduction of 100% during the 1st wk, falling to less than 60% after day 28, recovery by day 45, and remaining around 60% until day 90 with both doses. A similar pattern was observed for An. marajoara, whereas for An. triannulatus, the persistence remained around 80% for 90 days only at 3 g/m². With An. braziliensis, there was 100% persistence until day 14 with both doses, after which it fell abruptly and did not recover. This is the first time that B. sphaericus has been evaluated for these anopheline species in Venezuela, producing promising results for the national malaria control program.

Effectiveness and mass production of mermithid nematode parasites of mosquito larvae in Mexico

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Mermithid nematodes are under evaluation for the biological control of mosquito larvae in Mexico. Mass production of the mermithid nematodes, Romanomermis iyengari and Romanomermis culicivorax, was accomplished in a dedicated facility with the use of 500 rearing trays containing 1,000 Culex quinquefasciatus larvae per tray as the host species. Infection of mosquitoes, development of the mermithid parasitic phase, and emergence of the postparasites was completed every 10 days. The rearing system produced 360 cultures monthly, which, after maturation, yielded 150 million infectious nematodes for application to mosquito habitats of 50,000 m² (at an estimated concentration of 3,000 nematodes/m²). Applications of 3,000 preparasitic nematodes/m² were applied to mosquito larval habitats in the vicinity of malaria endemic communities on the coast of the state of Oaxaca, Mexico. Parasitism of mosquito larvae was evaluated at 3 days postinfection. The level of parasitism in Anopheles larvae varied from 46% to 100%, depending on the characteristics of the habitat. Thus, the mermithids proved to effectively reduce the population of mosquitoes transmitting malaria.

An efficacy evaluation of aerially applied Anvil 10+10⁵ in Valle de Banderas, Nayarit, Mexico

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An efficacy evaluation was performed in March 2008 to delineate the performance of Anvil 10+10⁵ when applied undiluted by air for adult mosquito control. The formulation was applied from a Piper Pawnee aircraft equipped with 2 Micronair AU 5000 rotary atomizers. All mosquitoes (Culex quinquefasciatus) utilized during the evaluation were supplied by the Medical Entomology Laboratory, University of Nuevo Leon in Monterrey, Mexico. Results indicated that aerial applications of Anvil 10+10⁵ (sumithrin) in Mexico can be effective through the utilization of proper equipment and calibration procedures, appropriate meteorological and mosquito species parameters, and application of formulations following label recommendations.

International public health pesticide perspectives: Current challenges and regulatory strategies for development of new pesticide tools

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From a global perspective, public health programs are faced with a depleting arsenal of effective insecticides. This is mainly due to the resistance of major vectors to common insecticides, but vector control options have been limited further due to the withdrawal or abandonment of certain pesticides for reasons of safety or high cost of registration and re-registration. Existing tools have been preserved through judicious use of insecticides based on integrated vector management (IVM) and insecticide resistance management (IRM). During this time, the pesticide regulatory landscape has become a more complex entity, research and development budgets for public health pesticides have nearly disappeared, and entomologists are rarer than many endangered species. The time for a global approach to development and registration of public health pesticides is at hand. Very few new products are likely to be developed by industry without harmonization of global- and country-level approaches to registration. Public health pesticide registration must be characterized by a strategy that creates a regulatory environment that promotes development of new public health pesticide uses. Approaches, ideas, and strategies for this approach will be discussed, as well as the upcoming “International Public Health Pesticides Workshop: New Approaches and Strategies for Development of Pesticide Tools,” which is to be held in London, United Kingdom, in May 2009.

Levels of insecticide resistance and resistance mechanisms in Aedes aegypti from Merida, Yucatan, related to 2 dengue transmission seasons

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Seasonality (rainy and dry seasons) exerts a direct influence on abundance of Aedes aegypti populations. Variables of warm and humid weather in the city of Merida, Yucatan, are related to the development of these mosquitoes, and their high numbers create a potential risk for dengue outbreaks in this city. Temephos as larvicide and permethrin for adult control are commonly used in Mexico, although fenothrin has also been used during outbreaks. For Yucatan State, the health department of Mexico reported 453 dengue and 99 dengue hemorrhagic fever cases for 2008. The goal of this study was to determine insecticide resistance levels for permethrin and fenothrin in adults and temephos in larvae. The samples were collected in rainy and dry seasons in Merida, Yucatan, in 2007. Bottle bioassays and biochemical tests were performed to determine resistance ratio (RR) and detoxifying enzymes. Results showed RR values for permethrin in rainy season of 30×, and the temephos RR value was 38×, whereas in the dry season, permethrin showed an RR value of 23× and temephos of 76×.

Resistance to pyrethroids in adult populations of Aedes aegypti from Veracruz, east coast of Mexico

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Aedes aegypti is the primary vector of dengue fever and dengue hemorrhagic fever (DHF) in Mexico. Although Aedes albopictus is present in many areas of the country, it has not yet been implicated as an important vector of dengue viruses. The coastal zones of the country are the most affected by this disease, and the state of Veracruz, with an area of 72,815 km² and coastline of 684 km, has had the highest number of cases of classic dengue and DHF in recent years relative to the national level. In 2007 alone, there were 14,099 cases, of which 1,962 were DHF, and through November 2008, there were 1,916 cases of classic dengue and 1,535 of DHF. The present study determined the levels of resistance to 8 pyrethroids in 7 adult populations of Ae. aegypti from the state of Veracruz. The resistance ratio (RR) was determined based on the susceptible New Orleans strain of Ae. aegypti. RR values were calculated based on KD50 (knockdown dose) with 1 h exposure, LD50 (lethal dose) with 1 h exposure and recovery at 24 h, in addition to KT50 (knockdown time) and LT50 (lethal time). In general, the results showed a greater susceptibility of the populations to 4th-generation pyrethroids in comparison to permethrin and fenothrin, for which the highest levels of resistance were found. The information generated by this work will be of great help for the selection of viable insecticides in a rotation program for the control of mosquito populations at the local/regional level.
Temephos and deltamethrin susceptibility and enzymes associated with resistance in *Aedes aegypti* from Mexico

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Larval and adult susceptibility to temephos and deltamethrin were evaluated in 16 subpopulations of *Aedes aegypti*. For larval bioassay, we recorded mortality at 24 h and obtained the LC50 and resistance ratio (RR) relative to New Orleans reference strain of *Ae. aegypti*. The CDC bottle bioassay was used for deltamethrin testing of female mosquitoes; RR values were calculated based on the KD50 (knockdown dose) with 1 h exposure, and LD50 (lethal dose) with 1 h exposure and recovery at 24 h. The LD50 for temephos varied from 0.012 µg/ml to 0.120 µg/ml with RR between 1.3× and 12×; KD50 and LD50 with deltamethrin varied from 0.041 µg/bottle to 0.237 µg/bottle (RR 2.1× to 12×) and 0.036 µg/bottle to 0.305 µg/bottle (RR 3.3× to 27×), respectively. Biochemical assays were performed to identify and document resistance related to enzyme quantification including: α and β esterases, glutathione s-transferases (GST), mixed function oxidases (MFO), and insensitive acetylcholinesterase (iAChE). The mean values were compared with those of the New Orleans strain, and they indicated significantly greater amounts of α, β esterases and GST in all subpopulations studied.

**Knockdown resistance (KDR) associated with permethrin resistance in *Aedes aegypti* from Guerrero State on the west coast of Mexico**

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In Mexico, permethrin is commonly applied as a mosquito adulticide as established by Official Mexican Standards for the monitoring and control of vectors (NOM-032-SSA2-200). Because it is widely used, resistance to this insecticide may become common in many mosquito populations, and monitoring will be crucial to ensuring effective vector control. Metabolic resistance and target site insensitivity are both major forms of pyrethroid resistance. “Knockdown resistance” (KDR) is a generic term applied to insects that fail to lose coordinated activity immediately following pyrethroid exposure. A new mutation (Iso1016) was found highly associated with knockdown resistance in *Aedes aegypti* from Isla Mujeres, south of Mexico (Saavedra et al. 2007). In the current research, we associated enzymatic resistance and Iso1016 in an *Ae. aegypti* strain from Zihuatanejo, Guerrero. KDR frequency of 0.49 was obtained, and 20% of the mosquitoes analyzed had high levels of detoxificative enzymes.

**The rise of a KDR mutation in *Aedes aegypti* in México**

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Pyrethroids are commonly used as mosquito adulticides, and evolution of resistance to these compounds is a major threat to public health. “Knockdown resistance” to pyrethroids (KDR) is frequently caused by nonsynonymous mutations in the voltage-gated sodium channel transmembrane protein (para) that reduce pyrethroid binding. Early detection of KDR is critical to the development of resistance management strategies for *Aedes aegypti*, the most important vector of dengue and yellow fever viruses. The present study analyzed the rise of KDR mutation over the past 7 years in 14 states of Mexico and determined that the KDR mutation has been increasing considerably, mainly in the state of Veracruz. Areas like Martinez de la Torre, Tantoyuca, and Poza Rica did not have KDR in 2000, but in 2007, KDR frequencies increased to 0.43, 0.38, and 0.70, respectively.

**Morphometric variation between insecticide-resistant and insecticide-susceptible populations of *Aedes aegypti* from 2 different eco-epidemiological regions of Colombia**

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*Aedes aegypti* is the main vector of dengue fever (DF) in Colombia. Although DF incidence increases throughout the country, some regions are considered hyperendemic, but the factors influencing this behavior have not been determined. Considering that the body size of the
mosquitoes reveals many bionomic factors, such as vector competence and response to insecticides, it is possible that morphological variation could be affecting the DF emergence and the insecticide resistance status of *Ae. aegypti*. Geometric morphometrics were used to evaluate morphometric variation of 7 *Ae. aegypti* populations collected in the municipalities of Quibdo (western Colombia, low incidence) and Cúcuta (eastern Colombia, high incidence), and a susceptible reference strain (Rockefeller). Sixteen landmarks were selected on the wings from digital pictures of 417 females, and these were digitized with coordinates. The coordinate matrix was processed by generalized Procrustes analysis, which generated size and shape variables free of nonbiological variation. Size and shape variables were analyzed by univariate and multivariate statistics. Both size and shape indicated a significant morphological difference related to the geographical location of populations and insecticide resistance status ($P < 0.0001$). However, this difference is more evident regarding the shape. These data delineated the existence of isolated populations and therefore different evolutionary paths, which may be associated with the ecological conditions, levels of insecticide resistance, and human elements. Complementary studies on the genetic structure are in progress.

**Field evaluation of mixture application of Vecto-Bac**\(^\text{TM}\) WDG (Bacillus thuringiensis var. israelensis) and Aqua K-Othrine\(^\text{TM}\) EW (deltamethrin) for simultaneous control of larval and adult *Aedes aegypti* and *Ae. albopictus* in critical spots in Tapachula, Chiapas, Mexico in 2008

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Empty lots, cemeteries, large refuse containers, tire dumps, junk yards, and closed or abandoned houses can be important contributors to *Aedes aegypti* and *Ae. albopictus* populations, offering breeding sites and resting places for adult mosquitoes that can often be difficult to access for routine larviciding operations in dengue control programs. We evaluated the application of a larvicide and adulticide mixture, using low-volume backpack sprayers: 125 g of VectoBac\(^\text{TM}\) WDG (Bacillus thuringiensis var. israelensis, 3,000 ITU/mg) plus 200 ml of Aqua K-Othrine\(^\text{TM}\) (deltamethrin 2.04%, with FFAST\(^\text{TM}\) technology) in 10 liters of water, to treat approximately 2,500 m\(^2\). A tire dump and a car junkyard in Tapachula, Chiapas, Mexico, were treated with the mixture. Pretreatment evaluation of 20 containers showed 100% infestation, with an average of 12.75 L\(_3\), 14.0 L\(_4\), and 4.95 pupae per container, and a landing rate of 12.5 female *Ae. albopictus* per h. One-day posttreatment, the larval and pupal counts and the landing rate were zero. At 25 days posttreatment, the container index was 5%, with an average of 0.3 L\(_3\) and 0.15 L\(_4\) and no pupae, and zero landing rate. This larvicide-adulticide treatment could offer a rapid and effective intervention measure to interrupt transmission in dengue outbreaks.

**Propagation of Lagenidium giganteum for control of *Aedes aegypti* larvae**

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*Lagenidium giganteum* has been used as a biological control agent because of its ability to be recycled at suitable levels and its relative ease of in vitro propagation. In this study, we tested 2 *L. giganteum* strains that were propagated on several liquid media. The medium composed of extract of ground sunflower seed produced the highest number of oospores after 6 days of fermentation at 25°C ± 2°C and 150 rpm agitation on a rotatory shaker, yielding 5.9 × 10\(^4\) oospores/ml and 5 × 10\(^4\) presporangia/ml. On the other hand, the culture medium including soy flour produced 0.85 × 106 oospores/ml and 7 × 10\(^4\) presporangia/ml under the same fermentation conditions used above. Both cultures were tested against 1st instar *Aedes aegypti* and produced 90.2% mortality at 48-h postexposure for cultures obtained in the sunflower seed medium, whereas the cultures using soy flour produced 92% mortality at 96-h postexposure. The LC\(_{50}\) for both cultures at 24-h posttreatment was determined: 39.9 µl/ml water for the sunflower seed culture and 315 µl/ml for the soy flour culture in bioassays against 1st instar *Ae. aegypti*.

**Evaluation of *Metarhizium anisopliae* and *Beauveria bassiana* strains for the control of *Aedes aegypti* larvae in the laboratory**

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Among the most important biological control agents for mosquitoes are Bacillus thuringiensis var. israelensis and B. sphaericus, although there are fungal pathogens that impact mosquitoes but have been less studied. In this work, we tested 3 strains of Metarhizium anisopliae and 2 strains of Beauveria bassiana against late 3rd instars of Aedes aegypti, which showed variable mortality. In the 1st bioassay, using the same conidia concentrations, the M. anisopliae (IB-Ma-2) and the B. bassiana (IB-Bb-2) strains showed significantly higher mortality percentages (64% and 50%, respectively) (F = 26.55, df = 4, P < 0.0001), while in the 2nd bioassay, only the M. anisopliae (IB-Ma-2) strain presented the highest mortality percentage (80%) (F = 70.62, df = 4, P < 0.0001). In the 3rd bioassay, the B. bassiana (IB-Bb-2) strain showed a significantly higher mortality percentage (39%) at 5-days posttreatment. New bioassays were performed to calculate the LC50 values. We found values of 2.45 and 1.47 × 10^6 conidia/ml for the M. anisopliae (IB-Ma-2) strain at 48- and 120-h posttreatment, respectively, whereas for B. bassiana (IB-Bb-2), we found values of 4.9 and 3.86 × 10^6 conidia/ml for 48- and 120-h posttreatment, respectively.

**Sensitivity of Aedes aegypti adults to the vapors of Eucalyptus essential oils and 1,8-cineole**

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Vapors of essential oils extracted from various species of Eucalyptus (E. gunnii, E. tereticornis, E. grandis, E. camaldulensis, E. dunnii, E. cinerea, E. saligna, E. sideroxylon, E. globulus ssp. globulus, E. globulus ssp. maidenii, E. viminalis, and the hybrids E. grandis × E. tereticornis and E. grandis × E. camaldulensis) and their major components were found to be toxic to adult Aedes aegypti. A 10-μl aliquot of each oil was placed in a cylindrical test chamber, and the number of knocked-down mosquitoes was recorded as function of time. Knockdown time 50% (KT50) was then calculated. Results showed that E. viminalis had a high knockdown activity of 4.2 min, in the same order as dichlorvos, a standard knockdown agent. A correlation was observed between the content of 1,8-cineole in the Eucalyptus essential oils and the corresponding toxic effect. Regression analysis also showed a statistically significant relationship between KT50 values and the inverse function of vapor pressures (1/Vp) of the different essential oil components. The results of the present study showed that 1,8-cineole and Euca-

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