MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—AN 18TH SYMPOSIUM

GARY G. CLARK¹ AND YASMIN RUBIO-PALIS²

ABSTRACT. The 18th Annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 74th Annual Meeting in Sparks, NV, in March 2008. The principal objective, as for the previous 17 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 35 presentations that were given orally in Spanish or presented as posters by participants from 5 countries in Latin America and Puerto Rico. Topics addressed in the symposium included surveillance, chemical and biological control, insecticide resistance, and repellency of Aedes aegypti; 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 Rhodnius.

KEY WORDS Mosquitoes, dengue, malaria, West Nile virus, mosquito control, bionomics, insecticide resistance, Aedes, Anopheles, Culex, Triatoma, Rhodnius

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes and other arthropods 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 promoted interaction with mosquito control industry representatives and professional colleagues in the USA who are involved in mosquito and vector control, training, and research at the university level, and with state and federal government officials.

This publication includes summaries of 35 presentations that were given in Spanish by participants from 5 countries in Latin America and Puerto Rico. Topics addressed in the symposium included surveillance, chemical and biological control, insecticide resistance, and repellency of Aedes aegypti; 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 Rhodnius. Summaries of 15 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).

SUMMARIES

Accurate, rapid, and robust sweep-net estimations of total immature Aedes aegypti
Claudia M. E. Romero-Vivas (clromero@uninorte.edu.co), Humberto Llinás, Andrew K. I. Falconar

The ability of a simple and rapid sweeping method, coupled with calibration, 4th instars (L3/L4) in large (20–6,412 liters) water storage containers at different water levels, in the 19–30°C dengue virus transmission–temperature range, was evaluated. Using this method, 3 calibration factors could accurately and robustly estimate total Aedes aegypti L. pupal numbers in their principal habitat from 14 to 1,630 m above sea level (19–30°C). New sets of calibration factors were needed, however, to accurately and robustly estimate Ae. aegypti L3/L4 larval populations in these water storage container types at each of 4 study sites at 14, 358, 998, and 1,630 m above sea level. Aedes aegypti L3/L4 larval collections, using this single water-surface-layer sweeping method, were less efficient than for pupae despite L3/L4 instars being present in greater numbers. Because this method was both rapid to perform and did not disturb the sediment layers in these domestic water storage containers, it was more acceptable by the residents and, therefore, ideally suited for routine surveillance purposes and to assess the efficacy of Ae. aegypti control programs in dengue virus-endemic areas throughout the world.

Efficient protein extraction in Aedes aegypti for proteome analysis
Flor Herrera (flormhq@gmail.com), Jose Rivero, Susana Zahalan, Nardy Diez, Yasmin Rubio-Palis
Insecticide resistance has a negative impact on the control of mosquito-borne diseases like dengue. Application of proteomics and protein identification methods, together with the completion of genome sequences for insects, give us the tools for examining insect resistance at a molecular level. We are interested in studying the proteins of *Aedes aegypti* from different regions of Venezuela with variable degrees of insecticide resistance. For this we extracted proteins from *Ae. aegypti* using Triton X-114 (Bordier C., JBC 1980, 256:1604–1607) to solubilize membranes and whole cells and to separate integral membrane proteins from hydrophilic proteins. Results showed that the quantity of proteins obtained was 8–10% of the total mosquito weight. The distribution of the proteins was 90% hydrophilic and 10% hydrophobic. The proteins were then separated by bidimensional electrophoresis, revealing the presence of approximately 60 and 30 spots for the hydrophilic and hydrophobic proteins, respectively. Financial support was provided by Consejo de Desarrollo Científico y Humanístico-Universidad de Carabobo (CDCH-UC) and the Fondo Nacional de Ciencia, Tecnología e Investigación (FONACIT), Venezuela.

**Dry-season investigation of *Aedes aegypti* in a dengue-endemic area**

Roberto Barrera (rbarrera@cdc.gov), Mary Hayden, Manuel Amador, Annette Díaz, Joshua Smith

We investigated *Aedes aegypti* (pupal survey, adults) in 2 neighborhoods with high (H) and low (L) dengue endemicity during the dry season of 2007 in San Juan, Puerto Rico (March–April), to determine if dengue endemicity was associated with the permanency of aquatic habitats and mosquito productivity. Our hypothesis was that dengue endemicity is due to the presence of containers with water during the low-rainfall season that are generated by humans (water storage vessels), climate (rainfall uniformly distributed in time), or both. We sampled mosquito pupae in 300 houses in each neighborhood. Adult mosquitoes were captured using 20 lured BG-sentinel traps for 2 wk (160 trap-days). We sequentially sampled the H neighborhood for 2 wk first, then the L neighborhood for another 2 wk. Heavy rainfall fell for 1 day during the 2nd week of the study, possibly affecting our measures of mosquito abundance in L. More pupae and pupae per container of *Ae. aegypti* were found in H (1,384; 2.2 ± 0.5) than in L (909; 1.1 ± 0.2). The mean number of *Ae. aegypti* pupae in household water-filled containers in H (2.9 ± 0.7) was larger than in L (0.8 ± 0.2), but the reverse was observed in rainwater-filled containers in H (0.7 ± 0.3) and L (2.7 ± 0.7). Significantly more adult *Ae. aegypti* were captured in L (1,633 females, 1,445 males) than in H (755 females, 423 males). This contradictory result is clearly the product of a single instance of heavy rainfall in L, where otherwise, based on immature indices, we expected lower adult *Ae. aegypti* productivity.

**The use of an ovitrap index to monitor *Aedes aegypti* in Belo Horizonte, Brazil**

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Since 2001, the Zoonosis Control Department (ZCD) in Belo Horizonte has subsidized vector control activities by providing ovitraps. The ZCD verified that this practice is very useful in the detection of new infestations and in monitoring *Aedes aegypti* adult populations. We analyzed data from January 2003 to December 2006. Belo Horizonte possesses 1,686 points where ovitraps are distributed in the 9 regions of the municipality. These traps are operated biweekly, maintaining a 200 m buffer radius. The 144,471 traps were operated and 47,721 presented eggs. The index of positivity of ovitraps and the index of density of eggs (IDO) varied by year from 55.42 to 69.11 and by week from 82.69 to 24.34, indicating great variation during the year. We observed greater predominance of *Ae. aegypti* than *Ae. albopictus* Skuse during the study. This method proved to be sensitive and economical in the detection of *Ae. aegypti*. In periods of low infestation where larval surveillance was less effective, the traps aided in a decisive way in the early discovery of new foci. The data generated support, in a routine way, the vector control and reinforce the efficiency of the method used.

**Laboratory evaluation of spatial repellency and irritability of permethrin for *Aedes aegypti***

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Dengue, a major public health problem throughout subtropical and tropical regions, is an acute infectious disease characterized by biphasic fever, headache, pain in various parts of the body, rash, lymphadenopathy, and leukopenia. *Aedes aegypti*, the primary vector of dengue and urban yellow fever, exhibits resistance to several insecticides, a situation that creates many problems for vector control programs in several countries. In the present research, the HITSS system was used; this system has a modular design that examines several aspects (contact irritation, spatial repellency, and toxicity).
Currently, there are few tests for evaluating contact irritation and spatial repellency that are produced by adulticides on mosquito behavior, with the exception of the test for penetrating response. Still, there is no standard system for assessing new chemical agents that affect adult mosquito behavior. The behavioral response of *Ae. aegypti* to several doses (0.025, 0.25, 2.5, and 25 nmol/cm²) of the topical insecticide permethrin was evaluated. At a concentration of 25 nmol/cm², 51.6% contact irritation was obtained; at 25 nmol/cm², 56.66% toxicity was observed; and for spatial repellency, the response was similar at all doses.

**Synergistic effect of S,S,S,-tributylphosphorotrithioate (DEF), piperonyl butoxide (PB), and permethrin in two subpopulations of *Aedes aegypti* from northeastern Mexico**

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The bottle bioassay was utilized to determine the modulating effect of S,S,S,-tributylphosphorotrithioate (DEF) or piperonyl butoxide (PB) in combination with the pyrethroid insecticide permethrin on F1 adult females of 2 subpopulations of *Aedes aegypti* collected in the metropolitan area of Monterrey, Nuevo Leon, Mexico. The optimal dose of each synergist was determined as the maximal dose that did not cause mortality. The results obtained from the bioassays were analyzed to determine in each population the LC50 of the insecticide alone and in combination with each of the synergists, as well as the toxicity ratio (TR). The LC50 of permethrin of Subpopulation 1 was 0.45 ppm (0.30–0.65 ppm), while for the combination with DEF, it was 0.032 ppm (0.014–0.060 ppm) with a TR of 14.06 ×. For Subpopulation 2, the LC50 of permethrin was 1.92 ppm (1.57–2.31 ppm), and for the combination with PB 0.31 ppm (0.21–0.48 ppm) with a TR of 6.19 ×. The magnitude of the TR indicates that esterases are important in the detoxification of permethrin and that the synergist DEF could be a good choice for formulating with permethrin.

**Evaluation of copepods for the control of *Aedes aegypti* in northern Colombia**

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Different species of copepods were collected from various types of natural freshwater bodies in Departamento del Atlántico (Colombia) to identify those with the highest predatory capacity for *Aedes aegypti* larvae in the laboratory and under semi-field conditions. Although we found 3 species already known for their capacity of controlling mosquito vectors—*Mesocyclops brasilius*, *M. aspericornis*, and *M. meridianus*—we also found 6 other species within this order about which little is known concerning their ecology and geographic distribution. The predatory capacities of these species were determined in the laboratory, using petri dishes of 55 mm in diameter and 14 mm high with 20 ml of dechlorinated water. Ten replicates of different numbers of L1 larvae per copepod were used: 5, 10, 20, 30, 40, and 50. Before each test, the copepods were left without food for 72 h. There were significant differences in the average number of larvae attacked (df1 = 2, df2 = 172, F = 68.4, P < 0.05), with *M. meridianus* as the species with the highest capacity (79%), followed by *M. aspericornis* (68%) and *M. brasilius* (37%). *Mesocyclops aspericornis* were applied in the field and currently are being evaluated. Results of the field studies will be discussed.

**Permethrin and temephos resistance in five populations of *Aedes aegypti* in southern Mexico**

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Five field populations of *Aedes aegypti* were collected in Quintana Roo, México. Ovitraps were set in 3 different places (Lagunitas, Antorchista, and Calderitas) in Chetumal and in 2 other municipalities (Solidaridad and Lazaro Cardenas) in Quintana Roo. F1 larvae and adult mosquitoes were tested for susceptibility based on LC50 and LC95 against temephos (98%; CHEM Service, West Chester, PA) and permethrin (98%; CHEM Service), the most common insecticides used for dengue control programs in Mexico. Resistance ratio (RR) values for temephos in *Ae. aegypti* populations indicated that populations of Calderitas (RR = 82×) and Lagunitas (59×) are highly resistant to this insecticide compared with the susceptible New Orleans strain. Other populations studied did not show resistance to temephos. Analyzing resistance enzymes, we found insensitive acetylcholinesterase (iAChE) as the main mechanism of temephos resistance. In contrast to temephos results, all populations studied showed low RR values for permethrin, indicating that we do not yet have resistance to this insecticide in these populations.

**Identification and distribution of the mosquitoes of northeastern Mexico**

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Mosquitoes are the most important arthropod vectors of diseases in the world, including malaria, dengue, lymphatic filariasis, and equine encephalitis, which are completely dependent on mosquito transmission. Knowledge of mosquito taxonomy is very important for development of effective control measures. We conducted a study to identify the different mosquito species of northeastern Mexico and report the distribution of species not yet reported from this region. Immature and adult mosquitoes were collected and transferred to the Medical Entomology Laboratory at the Universidad Autónoma de Nuevo Leon for identification using keys corresponding to the study area. A total of 57 mosquito species were collected. These included 7 Anopheles, 14 Aedes, 1 Coquillettidia, 13 Culex, 3 Culiseta, 1 Deinocerites, 1 Lutzia, 1 Limatus, 1 Mansonia, 1 Orthopodomyia, 6 Psorophora, 1 Sabethes, 2 Toxorhynchites, 3 Uranotaenia, and 2 Wyeomyia.

Resistance mechanisms to temephos in a population of Aedes aegypti from Peru

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A population of Aedes aegypti from Peru was studied for its susceptibility to the larvicidal temephos. The LC50 was calculated based on a 24-h exposure of late 3rd and early 4th instars. The dead and living individuals were separated after exposure to the LC50 and microassays were used to determine the activities of detoxifying enzymes including A-esterases, B-esterases, oxidases, glutathione-S-transferase (GST), acetylcholinesterase, and insensitive acetylcholinesterase. The results were compared with those obtained for the susceptible New Orleans (NO) strain, which was studied in the same manner as the Peruvian population. The LC50 of temephos for the population from Peru was 0.034 μg/ml and the resistance ratio based on the LC50 of the susceptible NO strain was 13.76. The enzymes that surpassed the tolerance threshold established by NO strain were A-esterases with 21.6% and GST with 96.67% (P < 0.05).

Entomological and social evaluation of dengue transmission in areas of high and low disease incidence in Medellin and Bello, Colombia

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Dengue is the main vector-borne viral disease in Colombia. Its transmission is determined mainly by the vector mosquito population and by dynamic, socioeconomic, and climatic conditions. The interaction of these factors leads to differences in disease incidence among localities. In this study, Aedes infestation indices were compared among localities of high and low incidence in Medellin and Bello. Productivity by container type was estimated and a knowledge, attitudes, practices, and beliefs survey about dengue prevention was conducted; 597 houses were visited and 5,557 containers were inspected. Entomological transmission risk was observed in high-incidence localities as well as in low-incidence ones. When areas of high incidence were compared, Bello displayed higher values in entomological indices in contrast to what was observed in Medellin. Discarded tires proved to be the most productive habitats. The social analysis performed found that 80% of study participants know that dengue was transmitted by A.e. aegypti; however, only 65% could identify containers as larval habitats. Research findings demonstrated that a close relationship between entomological indices and disease incidence is not occurring. This confirms that more accurate entomological indicators for dengue are needed. This study was supported by Inter American Institute for Global Change Research (Training Institute Seed Grant, Jamaica), Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología “Francisco José de Caldas” (COLCIENCIAS), and Universidad de Antioquia.

Evidence of vertical transmission of dengue virus in Aedes albopictus and A.e. aegypti in southeastern Mexico

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The potential of vertical transmission of dengue virus in Aedes albopictus and A.e. aegypti was explored in 3 endemic neighborhoods in Tapachula municipality in southeastern Chiapas state during a dengue outbreak in 2006. In this outbreak, there were 85 reported cases, of which we studied 48 cases of dengue classic and 6 cases of dengue hemorrhagic fever that were detected by the Ministry of Health’s epidemiological surveillance system. In each neighborhood, a random buffer of 200 m was randomly selected between 30 and 24 houses. Two plastic cans were placed in each house, which were changed every 5 days; positive cans were recorded and transferred to the insectary for emergence. The adult mosquitoes that emerged were groups of pools with 1 to 25 females and analyzed by reverse transcription-polymerase chain reaction (RT-PCR) technique to detect the dengue viral
Serologic evidence for West Nile virus activity in two areas of Chiapas, México

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West Nile virus (WNV) is a member of the Japanese encephalitis virus complex within the genus Flavivirus, family Flaviviridae. In Chiapas, a serologic survey of 200 domestic animals conducted during 2001 detected a single animal (cow) with WNV-neutralizing antibody, a result considered inconclusive because of the complexities of determining the prevalence of WNV in several communities from the “Lacandon Forest” and the “Encrucijada,” 2 natural protected areas in Chiapas. We sampled blood from 351 domestic animals, including 215 chickens, 50 turkeys, 44 cattle, 34 ducks, 4 horses, 2 geese, and 2 pigs, and tested them for WNV-specific antibodies using a blocking ELISA. Of 36 reactive sera, 19 samples were confirmed by plaque-reduction neutralization test, including 8 chickens, 5 cattle, 4 turkeys, 1 horse, and 1 goose. After combining results for all species tested, the serologic prevalence in domestic animals was 17% (6/31), 7% (4/56), and 5% (2/42), respectively. Seroprevalence was lower (X2 = 5.6, P = 0.03) on the Pacific coastal plain in the communities of Rio Arriba (6%, 6/94) and Zacapulco (1%, 1/120).

First West Nile virus isolations from mosquitoes in the Neotropics

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There is ample evidence of West Nile virus (WNV) circulation in the Caribbean and other tropical areas of Latin America. Virus identification in the region has been difficult because of an absence of patent epizootics or epidemics. The WNV surveillance in North America has used dead birds, horses, sentinel chickens, and mosquitoes. We are not aware of surveillance programs in tropical America using sentinel chickens coupled with mosquito trapping to detect active WNV transmission and isolate the virus. We initiated a sentinel chicken WNV surveillance program in July 2006 in eastern Puerto Rico, targeting areas where WNV antibodies had previously been detected in a resident bird and 3 horses in 2004. Surveillance was conducted by taking blood samples every 2 wk from 60 nonimmune chickens distributed across the 6 most representative terrestrial habitats. Blood specimens were tested by a chicken-specific IgM MAC-ELISA that would reflect a recent infection. The first positive chickens were detected in June 2007 in evergreen and mangrove forests. After this information was obtained, mosquito trapping was initiated around the sites where the cages were located and blood samples were taken weekly. Within 2 wk, WNV seroconversions were detected in all habitats. The WNV virus was isolated from Culex nigrripalpus and Cx. bahamensis and also detected in Cx. quinquefasciatus by RT-PCR. This is the first report of WNV isolations from mosquitoes in the Neotropics. We recommend sentinel chicken surveillance coupled with mosquito trapping to detect recent or active transmission of WNV in the tropics.

Culex mosquito distribution in Tecamac, Mexico State, Mexico

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West Nile virus (WNV) was initially isolated in America from Culex species and birds in the New York City area in 1999. Subsequently, the virus spread in the USA and many human cases have been reported. Culex mosquitoes are the most important vectors, although WNV has been isolated or detected in >20 mosquito species in the USA. West Nile virus was previously detected in equines and birds in Nuevo Leon, Tamaulips, Coahuila, and Yucatan states in Mexico. Because of the importance of this vector, the objective of the present research was to investigate the distribution of Culex species in Tecamac, Mexico State, Mexico, since the states of Mexico, Jalisco, Oaxaca, Michoacan, Tlaxcala, Saltillo, Nayarit, and Morelos are potential states where WNV can spread in Mexico. During May 2007, a total of 32 sites were sampled for mosquito eggs, larvae, and pupae. Culex quinquefasciatus Say was the dominant larval species collected. The likely
importance of this species in the natural history of WNV in the northeastern USA prompted us to evaluate the oviposition sites of Culex species and associated species as potential future foci of WNV transmission in Mexico. We have demonstrated the presence of Cx. quinquefasciatus in the State of Mexico.

Preferred indoor resting sites of Culex quinquefasciatus in Monterrey, Nuevo Leon, Mexico

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The study of adult Culex quinquefasciatus mosquitoes and the ambient dwelling factors explaining its presence are analyzed in this study. Description of this species’ preferred resting site was the goal of this work. Abundant Cx. quinquefasciatus populations are found in human settlements in Monterrey, northeastern Mexico. This fact represents a potential for West Nile virus (WNV) transmission, a disease with low endemicity to date but with the potential to cause large human outbreaks. Mosquito collections were made inside and outside houses, using a backpack aspirator. Several variables were recorded concurrently, such as resting site, height above the floor, wall material, and color, as well as blood digestion stages. The collected data were analyzed using the statistical program SPSS 10. Results showed a clear preference of this species to rest in bathrooms and bedrooms with smooth and rough wall surfaces. In addition, wall material was mostly dark-colored cement. The higher presence of resting mosquitoes was recorded when the wall had <50 cm height. Most of the resting sites had an average relative humidity of 50–60% and an average temperature of 30–35°C. The distribution of females according to blood digestion status showed that gravid Cx. quinquefasciatus preferred indoor resting sites. We concluded that indoor environment in Monterrey provided enough resources to allow this incriminated WNV vector species to rest in bathrooms and bedrooms with smooth and rough wall surfaces. In addition, wall material was mostly dark-colored cement. The higher presence of resting mosquitoes was recorded when the wall had <50 cm height. Most of the resting sites had an average relative humidity of 50–60% and an average temperature of 30–35°C. The distribution of females according to blood digestion status showed that gravid Cx. quinquefasciatus preferred indoor resting sites. We concluded that indoor environment in Monterrey provided enough resources to allow this incriminated WNV vector species to potentially maintain human epidemics.

Host-feeding preference of Culex quinquefasciatus in Yucatan State, Mexico

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Studies were conducted to determine the host-feeding preference of Culex quinquefasciatus in urban areas of Merida, Yucatan State, Mexico. Mosquitoes were collected in the backyards of houses, using wooden resting boxes. Collections were made 5 times per week from January to December 2005. DNA was extracted from engorged females and tested by polymerase chain reaction (PCR) using universal avian and mammalian-specific primers. DNA extracted from avian-derived blood was further analyzed by PCR using primers that differentiate among 3 avian orders: Passeriformes, Columbiformes, and Galliformes. Polymerase chain reaction products obtained from mammalian-derived blood were subjected to restriction enzyme digestion to differentiate between human-, dog-, cat-, pig-, cow-, and horse-derived blood meals. Overall, 82% of engorged mosquitoes had fed on birds and 18% had fed on mammals. The most common sources of avian-derived blood were Galliformes and Passeriformes. The most common sources of mammalian-derived blood were dogs and humans. The overall human blood index (HBI) was 6.7%. The overall forage ratio for humans was 0.1, indicating that humans are not a preferred host for Cx. quinquefasciatus in Merida.

Control of culicine mosquito larvae using micronized suspensions of calcium hydroxide

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Laboratory bioassays and preliminary field testing were conducted to evaluate the larvicidal effect of micronized suspension calcium hydroxide (the main component of lime) and the median lethal dose (LD₅₀) was determined. Mosquito larval 4th instars were kept in a mixture of drinking water and original pond water from selected locations (Mexico City for Culex spp. and Ayala City, Morelos State, for Aedes spp.). The control with Temephos® at 0.1 ppm (commercial concentration) caused 100% mortality of larvae 6 h after application and also killed pupae 28 h postapplication. The LD₅₀ for calcium hydroxide for Culex spp. larvae was 0.084% (w/v) 24 h after application; for Aedes spp. the CD₅₀ for larvae was 0.035%. The effects of calcium hydroxide on pupae showed much variation; therefore, we do not consider this compound effective against pupae. Preliminary field testing conducted in ponds in Ayala City indicate that calcium hydroxide seems an effective larvicide at LD₅₀. Our results show the potential of micronized suspensions of calcium hydroxide as mosquito larvicide. It is worth mentioning that lime is cheap and the people in the rural areas of Mexico and other countries are familiar with the handling procedures for this compound.
Brain cell karyotype of *Culex quinquefasciatus* at three temperatures from Colombia

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The objective was to analyze the chromosomes of *Culex quinquefasciatus* at 3 temperatures. The adults were collected in the following places: El Muña (elevation 2,565 m and 15°C average), Fusagasugá (elevation 1,700 m and 20°C average), and Girardot (elevation 289 m and 28°C average) in Cundinamarca Department, Colombia. Larval 4th instars were obtained from 1st generation of the 3 colonies. The larvae were placed in colchicine; the heads were then placed on microslide slides and the tissue was squashed. Forty slides were examined and mitotic chromosomes were photographed at 1,000×. The chromosomes were identified according to size and centromere position. The diploid number of *Cx. quinquefasciatus* was 2n = 6 for the 3 populations. The chromosome lengths were the following: pair I showed 2.82, pair II 4.02, and pair III 4.36 μm average at 15°C; pair I showed 3.39, pair II 4.57, and pair III 5.05 μm average at 20°C; and pair I showed 2.63, pair II 3.32, and pair III 3.85 μm average at 28°C. The chromosomal measurements showed a greater size in the chromosomes of *Cx. quinquefasciatus* from Fusagasugá although the differences were not statistically significant.

Life cycle of *Culex quinquefasciatus* at different temperatures in Colombia

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The objective of this study was to determine the life cycle of *Culex quinquefasciatus* at 3 temperatures. The adults were collected in the following places: El Muña (elevation 2,565 m and 15°C average), Fusagasugá (elevation 1,700 m and 20°C average), and Girardot (elevation 289 m and 28°C average) in Cundinamarca Department, Colombia. Laboratory colonies of this mosquito were maintained at 15°C, 22°C, and 28°C; according to origin. We observed that the mean life cycle of *Cx. quinquefasciatus* was 47.8 days at 15°C; while at 20°C, it was 29.4 days; and 35.9 days at 28°C. The life cycle of *Cx. quinquefasciatus* from El Muña (15°C average) was 38.9 days average at 22°C and 32.4 days average at 28°C; while mosquitoes from Fusagasugá (20°C average) showed a life cycle of 39.9 days at 15°C and 31.3 days at 28°C; and *Cx. quinquefasciatus* from Girardot (28°C average) showed a life cycle 41.6 days at 15°C and 28 days at 22°C. The differences in life cycle among the 3 populations of *Cx. quinquefasciatus* were statistically significant. The results suggest a good capacity of adaptation by *Cx. quinquefasciatus* to environmental changes.

Observations on the bionomics and ecology of *Anopheles (Nyssorhynchus) marajoara* in Venezuela

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*Anopheles (Nyssorhynchus) marajoara* Galvão and Damasceno has a wide geographic distribution in Venezuela. To contribute to the knowledge on the bionomics and ecology of this malaria vector, studies were conducted to identify and characterize larval habitats. Collections of adult females from light traps and on human baits were carried out in different localities in the states of Apure, Amazonas, Barinas, Bolivar, Delta Amacuro, and Guárico. This species was found at elevations between 35 and 220 m, in different ecosystems such as rain forests, gallery forests, savannahs, secondary vegetation, and rice, plantation, and cotton plantations. Field-collected females laid a mean of 78 eggs; the development from egg to adult takes 10 days at 28°C. Larvae were collected mainly in stream margins and semipermanent lagoons associated with *An. nuneztovari*, *An. argyritarsis*, *An. darlingi*, *An. braziliensis*, *An. oswaldoi*, *An. strodei*, *An. costae*, and *An. punctimacula*. *Anopheles marajoara* is more abundant during the rainy season (May–December), with a peak in August. Regarding the diel biting pattern, this species bites throughout the night indoors and outdoors, although significantly more mosquitoes were collected outdoors (>70%) and 75–83% mosquitoes were caught before midnight. The higher adult mosquito densities were found in Guárico State in sites close to rice fields.

Genetic structure of *Anopheles (Nyssorhynchus) marajoara* in Colombia

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Five *Anopheles marajoara* populations, representing diverse ecological conditions, were sampled throughout Colombia and analyzed using 9 variable DNA microsatellite loci. The overall genetic diversity (H = 0.58) was lower than that determined for some Brazilian populations using the same markers. The Caquetá population in Colombia had the lowest genetic diversity (H = 0.48) and it was the only population at Hardy–Weinberg equilibrium. Hardy–Weinberg disequilibrium in the remaining 4 populations was probably due to the Wahlund effect. The assignment analyses showed 2 incompletely isolated gene pools separated by the Eastern Andean cordillera. However, other possible geographical
barriers (rivers and mountains) did not play any role in the moderate genetic heterogeneity found among the populations (F_{ST} = 0.069). These results are discussed in relation to An. marajoara’s possible role as a malaria vector.

**Efficacy and persistence of methoprene on Anopheles albimanus pupae**

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Anopheles albimanus Wiedemann has been considered under natural conditions as human malaria’s principal vector in Central America. Methoprene, a synthetic juvenile hormone mimic, is used in the larval control of vector mosquitoes. This research contemplates the realization of a series of experiments under laboratory conditions with a granular formulation of methoprene (Altosid-G® 1.5%) and An. albimanus larvae to calculate the lethal concentrations of the product, as well as evaluate its efficacy and persistence. The aim of this study was to evaluate the efficacy and persistence of lethal effect of methoprene on An. albimanus pupae and to calculate the lethal concentrations (LC_{50}, LC_{95}, LC_{98}) of the product under laboratory conditions. The product’s persistence over An. albimanus colonized larvae was evaluated using the LC_{95} of 0.1515 ppm, at 0, 7, 15, 30, and 60 days posttreatment. According to the results obtained at 0 and 7 days posttreatment, the product’s efficacy was very satisfactory, with 95% and 75% mortality. But the percentage of dead pupae in treated water diminished at 15, 30, and 60 days posttreatment. In conclusion, this granular formulation has a short residual activity on An. albimanus pupae and the permanence of the product in treated water (15, 30, and 60 days) negatively affected its effectiveness.

**Isolation and selection of stocks of fungi for Anopheles albimanus mosquito control**

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Recently, 2 studies demonstrated that entomopathogenic fungi can kill adult anopheline mosquitoes and open new opportunities in the fight against malaria. The present study was carried out to isolate, identify, and select entomopathogenic stocks from native fungi associated with Anopheles albimanus. The isolations were made from water, ground, and larval samples collected in mosquito oviposition sites in the southeastern coastal plain of Chiapas, Mexico. A total of 29 stocks were isolated; the most frequent were Aspergillus niger (21.08%), Penicillium spp. (17.69%), Gliocladium spp. (8.62%), Aspergillus terreus (8.31%), Geotrichum spp., and Rhizopus spp. (5.38%). The native stocks of Gliocladium spp., in addition to Beauveria bassiana and Metarhizium anisopliae, were evaluated for pathogenicity on An. albimanus 3rd instars. Stocks of M. anisopliae showed the highest pathogenicity, causing 100% mortality during the first 24 h of exposure, to a concentration of 2.58 × 10^7 conidia/ml. Gliocladium spp. and B. bassiana showed 58.7% and 33.3% mortalities, respectively, until 7 days postinoculation. Stocks of M. anisopliae and Gliocladium spp. will be reactivated and reevaluated on mosquitoes to determine lethal concentration (LC_{50} and LC_{90}) because they are good candidates for biological control of mosquitoes.

**Quantification of Bacillus sphaericus produced by single larvae of Anopheles marajoara exposed to Vectolex WDG**

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Anopheles marajoara larvae killed by application of Bacillus sphaericus Neide (Vectolex® WDG 0.5; 1 and 1.5 kg/ha, Serotype H5a5b, strain 2362, 51.2%, 650 ITU/mg) were washed with a germicidal product to eliminate any traces of B. sphaericus on the external surface of the body. The larvae were placed individually in petri dishes containing 20 ml of distilled water, replicated 10 times for concentration. In the 1st 6 h and 12 h, samples were taken from 10 µl of each replication, which were inoculated in Lennox LB medium agar, incubated at 37°C for 12 h. Bacterial growth was observed with an uncountable number of colonies; subsequently at 18 h samples of dilutions of each medium, 1 × 10^{-2}, 1 × 10^{-4}, and 1 × 10^{-6}, were inoculated. We found that for the first 2 dilutions the number of colony-forming units was not quantifiable, while for dilution 1 × 10^{-6} showed bacterial growth with an average of 52 colonies/plate, which translates into 5,200/ml. Sporule and non-sporule forms in the colonies isolated by smear were observed. This confirms the reproduction of B. sphaericus in an aquatic environment.

**Elevated oxidase and esterase levels associated with DDT and lambdacyhalothrin cross-resistance in Anopheles darlingi from the Medio Atrato region of Colombia**

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Currently, indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are the mainstay in malaria prevention. These strategies use pyrethroid insecticides. In the Medio Atrato region of Colombia, IRS using lambdacyhalothrin is the main control measure. Earlier studies reported
Insecticide resistance evaluation of the main malaria vectors in Colombia

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After the ban on DDT usage and the decentralization of the malaria program in Colombia, different pyrethroid and organophosphate insecticides have been used for malaria control. Few local studies have been conducted of the insecticide susceptibility of the main malaria vectors. The purpose of this work was to update the susceptibility status of the 3 main vectors to these insecticides and to implement a national surveillance network of insecticide resistance. Three research institutes and entomology teams of 12 states were involved in the project. Susceptibility tests were carried out using both the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) methodologies. The insecticides evaluated were deltamethrin, lambdacyhalothrin, permethrin, cyfluthrin, DDT, fenitrothion, malathion, propoxur, bendiocarb, methyl-pirimiphos, and etofenprox. Out of 6 populations of *Anopheles albimanus* evaluated, 1 showed results compatible with resistance to lambdacyhalothrin. *Anopheles darlingi* showed resistance to DDT and lambdacyhalothrin in 1 population out of 9 evaluated, and *An. nuneztovari* showed resistance to deltamethrin, lambdacyhalothrin, DDT, and fenitrothion in 2 populations out of 4 evaluated. The resistant populations coincided with the regions with higher annual parasite rates and, therefore, extensive use of insecticides for malaria control. Local entomology teams were trained to continue the surveillance, and a national network for insecticide resistance of malaria vectors in Colombia was initiated. Financial support was provided by the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología “Francisco José de Caldas” (COLCIENCIAS).
It is well known that repellents do not kill mosquitoes; nevertheless, the best products provide long-lasting protection with just 1 application. Laboratory tests were made to compare the efficacy of 3 common commercial formulations, at both low and high concentrations: deet (7% and 25%), picardin (5% and 12%), and citronella (5% and 10%). The efficacy of these was tested against laboratory strains of Aedes aegypti. Feeding behavior of female mosquitoes was observed following application of each product. Differences were noted between the formulations tested and at different concentrations. An extended-duration repellent formulation containing 25% deet repelled significantly more than citronella but not compared with picardin at 12%, with the advantage that the latter causes less irritation for human skin.

Search for mutations in the super-kdr region of para in Aedes aegypti from Latin America

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Pyrethroid resistance in Aedes aegypti is increasing worldwide, and an insensitive sodium channel is likely to be the major mechanism of resistance. Most of the point mutations conferring knockdown resistance in the insect sodium channel gene occur in hydrophobic segment 6 of domain II of para and are postulated to prevent the rapid paralytic and lethal actions of all known pyrethroids but do not diminish the efficacy of other insecticides. The hydrophobic segment 5 of domain II of para is called super-kdr and can also carry mutations that confer much greater resistance to DDT and some pyrethroids. We screened exon 19 of para in Ae. aegypti that encodes hydrophobic segment 5 of domain II. We examined 1,110 mosquitoes in 37 strains from Latin America and found a transversion in the 2nd position of Leu946 that instead encodes Gln946. We developed a melting-curve single nucleotide polymorphisms–polymerase chain reaction (SNP-PCR) assay for these mutations that can be read either on an agarose gel or a melting curve. These results will provide new insight into the mechanisms by which pyrethroids modify the function of voltage-sensitive sodium channels.

Biochemistry and molecular characterization of the resistance to organosynthetic insecticides by Anopheles aquasalis from Río de Agua, Sucre State, Venezuela

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The municipality of Libertador, Sucre State, in northeastern Venezuela is known as a zone of coastal malaria where the principal vector is Anopheles aquasalis. A focal study of insecticide resistance was conducted to determine metabolic resistance mechanisms. Field populations of An. aquasalis were collected from several localities in Río de Agua. In the laboratory, we used bioassays and biochemical and molecular tests to identify and document resistance. High levels of alpha- and beta-esterases, glutathion-s-transferases (GSTs), and inhibited acetylcholinesterase (Ache) activity were detected. The absorbance values recorded were between 0.8 and 3.5 for esterases; between 0.19 and 0.32 for GSTs; and 52.2% for Ache. Mixed function oxidase is not operating because 93.3% of mosquitoes had reference values of <0.5 absorbance. Preliminary results with PCR have detected a kdr mutation in the Vgsc gene in mosquitoes studied in the locality. Research on An. aquasalis resistance is in progress to improve malaria vector control program in Sucre State and to implement resistance management strategies.

A historical review of Anopheles (Nyssorhynchus) darlingi distribution in the Peruvian Amazon

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Anopheles (Nyssorhynchus) darlingi [Root 1926] is one of the major vectors of malaria in the Americas and as such has become a focus of medical entomology research. Raymond C. Shannon is credited with first having documented the species in Peru from specimens collected in 1931 on the Peruvian–Brazilian border. While the original documented collection can be pinpointed, there is a great amount of uncertainty in regard to the past (and present) distribution of this species in Peru. Records indicate the possibility that An. darlingi is a relatively new species inside the areas of the Amazon region and that in recent years has greatly expanded its range throughout the Amazon. A thorough review was conducted on all available literature and records to determine past and present distribution within the Peruvian Amazon. Special attention was paid to documenting “nonliterature” sources such as personal communications, internal reports, and conference presentations so that all information up to this date could be accounted for in 1 publication. Speculation is provided as to the reasons responsible for the apparent dramatic distributional increase of this species in the Peruvian Amazon.
Use of Google Earth for the management of vector-borne diseases in resource-poor environments

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The operational value of a decision support system (DSS) for vector-borne disease management is enhanced by a geographic information system (GIS) spatial backbone allowing for visualization of spatiotemporal vector and disease patterns. Resource-poor environments in desperate need of GIS-based solutions for more effective disease management can be faced with the reality that even the most basic GIS data (e.g., administrative boundaries or city structure) are lacking. To address this problem, we have developed methodology for using free mapping tools to generate basic GIS data layers at minimal cost and serve as the spatial underpinning of a DSS for vector-borne disease management. Two cities in Mexico were used to demonstrate that a basic representation of a city useful as the spatial backbone in a dengue DSS can be developed at minimal cost from satellite imagery accessible through a free mapping tool (Google Earth). A stand-alone management tool for extraction of information from a data warehouse and generation of text-format reports and Google Earth-based map outputs also was developed. As a conclusion, the combination of free mapping tools and free or low-cost GIS software has tremendous potential for use in DSS to facilitate control of vector-borne diseases.

Educating children—a method for controlling triatomines
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In México, control of triatomines mainly relies on the use of insecticides, especially pyrethroids. Besides that method, physical improvement of human dwellings have been carried out in some places of México; however, in some cases, inhabitants of those towns have provided only limited participation, and the populations of bugs have not decreased. Taking into account those considerations, research involving children from 3 to 5 years old was recently developed in a city in the Jalisco State in western México. In that place, 2 kindergartens, in 2 similar neighborhoods (colonias) but far from each other, were selected. Entomological indices were recorded before intervention in each study areas. Children in one of the kindergartens were “trained” on Triatominae control by a projection of a series of images related to triatomines (e.g., their shelters); after that they were trained “in collection of triatomines.” Finally, as reinforcement, children were asked to draw those items that were more interesting to them. Meanwhile, the 2nd group was the untreated control. Post-intervention entomological indices were lower in the intervention area than in the control, apparently because the “open mind” of younger children let them receive information more easily.

Factors affecting the effectiveness of insecticides under field conditions to control Rhodnius prolixus
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An investigation of observational type was designed in 8 villages in Barinas State, Venezuela. The effectiveness of 10% lambdacyhalothrin (WP) 25 mg/m² and fenitrothion 40% (WP), 2 g/m² was measured in function of the mortality of the exposed triatomines using the World Health Organization (WHO) method. The susceptible “Chagas” strain of Rhodnius prolixus Stal was used. Houses were evaluated from the 1st day post-spray and then at a monthly frequency. We used the segmentation analysis in which the dependent variable was mortality. It showed that the 2 predictors that produced bigger discrimination and that were significant: time of action and surfaces. There was 100% mortality for the first 3 to 30 days post-spray; the surfaces presented mortality of 86.57% at 80 days and the surfaces of wood, cement, and painting wattle and daub presented 31.71%. At 90 days the surfaces of wood, cement–lime, and wattle and daub–lime presented mortalities between 11.11% and 62.96%. There are no reasons to suppose that the mortality is due to differences in the effectiveness of the insecticides. We demonstrated the interaction between the dependent variable (mortality) and the predictors of time of action and surfaces. There was no significant association in the predictors of temperature and RH with Pearson’s correlation (temperature 0.1580 and RH −0.0191).

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REFERENCES CITED