

MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A FOURTEENTH SYMPOSIUM

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ABSTRACT. The 14th annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 70th Annual Meeting in Savannah, GA, in February 2004. The principal objective, as for the previous 13 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 7 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies; studies of insecticide resistance; and population genetics, molecular, ecological, and behavioral studies of vectors of dengue (*Aedes aegypti* and *Aedes albopictus*) and other arboviruses, malaria (*Anopheles albimanus* and *Anopheles pseudopunctipennis*), leishmaniasis (*Lutzomyia*), and Chagas disease (*Triatoma*).

KEY WORDS Mosquitoes, West Nile virus, dengue, malaria, mosquito control, bionomics, *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Ochlerotatus*, *Lutzomyia*, *Triatoma*

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes, other vectors, 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 1st at the AMCA Annual Meeting in 1991 and at all subsequent meetings. In addition to providing a forum for scientists whose 1st language is Spanish, the session promoted interaction with mosquito control industry representatives and interaction with professional colleagues in the USA who are involved in mosquito vector control, training, and research at the university level, and with state and federal government officials.

This publication includes summaries of 37 presentations that were given in Spanish by participants from 7 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies; studies of insecticide resistance; and population genetics, molecular, ecological, and behavioral studies of vectors of dengue (*Aedes aegypti*) and other arboviruses, malaria (*Anopheles albimanus* and *Anopheles pseudopunctipennis*), leishmaniasis (*Lutzomyia*), and Chagas disease (*Triatoma*). Summaries of 11 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 Martínez 2001, 2002).

Financial support for this session was provided by the following sponsors: Syngenta, Valent, Wellmark, and the Pan American Health Organization. Funds from these sponsors provided Annual Meeting registration and partially defrayed travel expenses for many symposium participants. Enthusiasm and interest for this symposium among Spanish- and non-Spanish-speaking participants was high and it will continue to be part of future meetings.

SUMMARIES

Epidemiology of West Nile virus in northern Mexico in 2003

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West Nile (WNV) began to disperse in Mexico in 2003. Here, we report findings of an avian and equine surveillance program conducted in north-eastern Mexico. Bird collections from October 2002 to October 2003 totaled 737 individuals. The 1st occurrence of WNV-seropositive birds was made in February 2003 in the northern part of the state of Nuevo Leon. Two migratory species (*Zonotrichia leucophrys* and *Spizella pallida*) and 1 resident species (*Passer domesticus*) tested positive to 1112G WNV monoclonal antibodies by blocking enzyme-linked immunosorbent assay. In March in Tamaulipas State, 3 sampling areas (El Carrizal, in the city of Matamoros located near Brownsville, TX; San Fernando; and La Pesca) yielded seropos-

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itive birds. West Nile virus-positive bird sera also were collected in La Escondida in Coahuila State in May. Again in mid-summer, we found seropositive birds in the suburbs of the city of Monterrey (population 4 million). Overall, of 737 birds collected in the 3 states of northeastern Mexico, 20 (2.7%) were seropositive, whereas 6 (54.5%) out of 11 localities yielded seropositive birds. Reports from the Nuevo Leon State Health Department yielded only 1 seropositive human case in August 2003. Reports of dead and sick WNV-infected horses increased in spring and summer of 2003 in Nuevo Leon State.

Detection of dengue viruses in field-caught *Aedes aegypti* (Diptera: Culicidae) in Maracay, Aragua State, Venezuela, by type-specific polymerase chain reaction

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In a prospective field study conducted from November 2000 to December 2001, adult female *Aedes aegypti* were caught from several municipalities with high dengue (DEN) transmission in Maracay, Aragua State, Venezuela, and were tested for dengue viruses (DEN-1, DEN-2, DEN-3, and DEN-4). Virus detection was carried out by using reverse transcription polymerase chain reaction (RT-PCR). We analyzed a total of 296 pools of *Ae. aegypti* ($n = 1,632$ mosquitoes). From these, 154 pools ($n = 469$ mosquitoes) were collected from houses in which there were dengue-infected residents ("dengue case") and 142 pools ($n = 1,163$ mosquitoes) were from the houses surrounding the dengue case ("neighbor"). From the dengue case group, 8 mosquito pools (5.2%) were positive for dengue viruses, including DEN-1 (0.8%), DEN-3 (3.2%), and DEN-4 (1.2%). From the neighbor group, 18 mosquito pools (12.7%) were positive for dengue viruses, including DEN-3 (11.9%) and DEN-4 (0.8%). From these 26 RT-PCR-positive mosquito pools (containing 1–25 mosquitoes each), 22 pools (84%) were positive for DEN-3 virus. The most prevalent serotype in the 2001 dengue outbreak also was DEN-3. Virological surveillance of dengue viruses in natural populations of *Ae. aegypti* by RT-PCR could provide a powerful tool to assist in the control of dengue in this hyperendemic region in Venezuela.

Climate variability and dengue in a hyperendemic area of northern Venezuela

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Dengue is a major public health problem in Venezuela. The situation has worsened in recent years (e.g., in 2001, a total of 83,180 cases of classic dengue, 6,541 of dengue hemorrhagic fever [DHF], and 5 deaths were reported). To establish the population pattern of *Aedes aegypti* in relation to rainfall, a longitudinal study was conducted between January and December 2001 in the metropolitan area of Maracay, Aragua State. Adult mosquitoes were collected monthly inside houses by using a backpack aspirator. Climatological data (rainfall) were obtained from the Venezuelan Air Force Base in Maracay. A total of 845 houses in 87 barrios were visited, and 80.4% (679) of the houses were positive for *Ae. aegypti*. Vector abundance varied from 3.6 *Aedes*/house during April (at the end of dry season) to 14.7 *Aedes*/house during August (the peak of rainy season). Positive significant correlations were found between monthly rainfall and the number of *Aedes* per house ($P = 0.01$), the number of classic dengue cases ($P = 0.006$) and the number of DHF cases ($P = 0.02$), as well as the number of dengue cases (classic and DHF) and abundance of *Ae. aegypti* ($P = 0.0001$). Examination of the results of the Spearman correlation analysis suggested that rainfall positively influenced the abundance of *Ae. aegypti*, which resulted in an increase of the number of cases of dengue and DHF. Rainfall may be used as an indicator to apply effective and efficient vector control measures in Maracay.

A survey of anopheline species and *Plasmodium* infectivity in the Peruvian Amazon

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In 2001–02, 100 localities in the departments of Loreto, Ucayali, San Martín, and Madre de Dios, Peru, were surveyed for anophelines utilizing human-baited collections. A total of 86,267 anophelines were collected from 89 localities, including *Anopheles benarrochi*, *An. darlingi*, *An. mattogrossensis*, *An. triannulatus* s.l., *An. rangeli*, *An. oswaldoi* s.l., *An. nuneztovari*, *An. forattinii*, *An. intermedius*, *An. sp. near fluminensis*, *An. peryassui*,

An. nimbus, and *An. deaneorum*. Of these, 84,654 (8,815 pools) were tested by enzyme-linked immunosorbent assay for the presence of *Plasmodium* circumsporozoite (CS) proteins, but only 2 species, *An. benarrochi* and *An. darlingi*, were found to be positive. In *An. darlingi*, *P. falciparum* CS proteins were found in 0.7% of the 1,422 pools and *P. vivax* 210 CS proteins were found in 0.28% of the pools. Of the 6,323 *An. benarrochi* pools, 0.08% were positive for *P. falciparum* CS proteins, 0.032% were positive for *P. vivax* 247, 0.016% were positive for *P. vivax* 210/247, and 0.016% were positive for mixed *P. falciparum* and *P. vivax* 247 CS proteins.

Effect of temperature on the developmental cycle, life expectancy, and survivorship of *Anopheles albimanus* (Diptera: Culicidae) related to an El Niño event

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In Colombia, a strong association has been observed between malaria incidence and temperature during El Niño events. Climatic changes might alter the vector population dynamics and therefore increase malaria transmission. To evaluate the influence of temperature on development cycle, life expectancy, and survivorship of *Anopheles albimanus*, the following study was performed under controlled laboratory conditions. The study was conducted at 4 different temperatures (i.e., 24, 27, 30, and 33°C). For each selected temperature, the development cycle, life expectancy, and survivorship were evaluated in 4 cohorts of 100 1st instars each. Examination of results showed that increased temperatures reduced the development cycle, but no significant differences were observed at the 2 higher temperatures. Similar results were obtained for survivorship. Life expectancy was reduced when mosquitoes were incubated at 33°C, in contrast to mosquitoes incubated below 30°C, where no significant differences were observed. Taking these results all together, we hypothesize that during El Niño events, increased temperatures produce a reduction in the larval development without affecting the life expectancy. This allows the vectors to live longer in the adult stage, therefore enhancing the probability of more infective bites and thus increased malaria transmission.

Seasonal study of containers with *Aedes aegypti* (L.) in Nuevo Leon during 2000–02

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Breeding sites of *Aedes aegypti* were analyzed from 2000 to 2002 in the metropolitan area of Monterrey. Containers were classified as "controllable," "destructible," and "controllable with temephos" in order to determine the most permanent breeding sites (deposits) present. During 2000, the percentage of positive containers was 2.6%. When this value was considered as 100% and analyzed, 23.3% of the containers were controllable, 25.3% were destructible, and 51.3% were controllable with temephos. In 2001, the percentage of positive containers was 2.5%. Of this total, 25.6% were controllable, 21.6% were destructible, and 51.4% were controllable with temephos. In 2002, the percentage of positive containers was 2.7%. These data include 25% controllable, 23% destructible, and 52% controllable with temephos. The most common positive containers during the 3 years of study were those where larvicide was used.

Comparison of *Aedes aegypti* density indices with the currently used larval indices

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The aim of this study was to compare density indices of *Aedes aegypti* obtained in a dengue fever (DF) endemic area where the most abundant and productive vector breeding sites were located indoors. One hundred twenty premises in an urban area of Colombia where DEN-1 and DEN-2 viruses cocirculated were randomly selected and sampled for 7 months. The geometric mean monthly numbers (density index [DI]) of eggs of *Ae. aegypti* (ODI), 4th-stage larvae (LDI), pupae (PDI), and adults (ADI) were calculated based on the use of ovitraps, nets, and manual aspirators, respectively. A negative temporal correlation was observed between the LDI and the ODI ($r = -0.83$, $df = 5$, $P < 0.01$). Positive temporal correlations were only observed between the LDI and the PDI ($r = 0.90$, $df = 5$, $P < 0.05$) and the Breteau and house indices ($r = 0.86$, $df = 5$, $P < 0.01$). No other correlations were found between these indices and any of the other density indices or the incidence of suspected DF cases in residents, the temperature, the rainfall, or seasonal fluctuations. Therefore, our re-

sults probably were due to the most productive breeding sites of *Ae. aegypti* (large water containers) being located indoors in this study area.

Using ecological niche modeling in entomological supervision of *Aedes aegypti* in Mexico

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Epidemiological surveillance systems for emerging infectious diseases involve monitoring of both humans and vectors. Vector sampling is important for determining spatiotemporal dynamics of their populations, with the goal of designing prevention and control strategies or emergency responses. Traditional entomological surveillance programs for *Aedes aegypti* in relation to dengue and dengue hemorrhagic fever include mosquito and larval sampling in both domestic and peridomestic environments. This information nevertheless generally lacks wide geographic and temporal coverage, which limits the ability to efficiently respond to potential outbreaks. New approaches for modeling distributions of species based on their ecological niches have proven powerful and effective in predicting species' geographic distributions, even with relatively sparse and scattered occurrence data. We evaluated the use of occurrence data for *Ae. aegypti* obtained from traditional sampling methods, coupled with satellite imagery and additional environmental data, to produce time-specific distributional models for this species by using ecological niche modeling to identify areas of potential risk of infection of diseases transmitted by this vector in Mexico.

Preliminary evaluation of pupal collecting methods in drums in Maracay, Venezuela

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Sampling of aquatic stages of *Aedes aegypti* has made valuable contributions to the development of vector control methods. Mosquito surveillance has greatly improved and there is a permanent concern that it is necessary to evaluate the productivity of all containers. Moreover, the pupal survey is an im-

portant tool that helps evaluate containers, revealing important information about the specific risk container as a function of vector abundance. To measure the productivity of containers, sampling methods for pupal collections were carried out in the laboratory by using 200-liter drums. Three strategies for collecting pupae (A, C, and H), each with 3 replicates for each method on 5 different days were tested. Between 100 and 400 immature stages were released into full water drums. Significant differences between methods were found ($P < 0.001$; Duncan's multiple test). Additionally, the percent recovery for immature stages for each sample method was determined as 93% for strategy "Abel" (A), 56% for "colador" (C), and 71% for "hamaca" (H). The mean of percent recovery showed significant differences among methods A and H compared with C ($P < 0.05$). The number of immersions were counted in 1 min, with the mean of A = 1, of C = 6, and of H = 4. In view of these results, a survey method involving the counting of pupae in a field test is needed to evaluate containers as useful tools for planning vector control programs.

Estimation of gonotrophic cycle length, survival rate, and host-seeking behavior of *Aedes albopictus* in southern Mexico

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Recently, *Aedes albopictus* (Skuse) was reported from Chiapas, Mexico. The rapid expansion of the natural range of *Ae. albopictus*, along with population growth, has caused concern because of its potential as an arbovirus vector. Little information is available concerning the reproductive biology and behavior of this species in this region. Here, we present an evaluation of a time-series technique to estimate the length of the gonotrophic cycle and survival of host-seeking populations of *Ae. albopictus* collected in southern Mexico. Diel collections were carried out by using human bait over a 21-day period in a cemetery located along the perimeter of Tapachula, Chiapas. The gonotrophic cycle was estimated to be 4 d and the daily survival rate was estimated vertically to be 0.96 (daily parity rate = 0.85 for a 4-day gonotrophic cycle of 0.961/4), according to Davidson's equation. Further, we determined that the host-seeking activity was bimodal, with the 1st peak between 0800 and 1000 h and a 2nd peak between 1400 and 1600 h.

Integrated control of *Aedes aegypti* and *Ae. albopictus*: a progress report

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Current dengue vector control efforts fail to suppress mosquito populations and cannot prevent dengue transmission. The causes for this situation include limited resources, defective logistics, poor training of operational personnel, null community participation, and favorable ecological conditions that promote abundant mosquito populations and circulation of dengue viruses. This ongoing study aimed to develop and evaluate dengue vector integrated control strategies compared to current approaches in several small to mid-sized cities in southern Chiapas, Mexico. Treatments were antilarval measures with no insecticide, including community education and participation (CP) + complete adulticide measures, including indoor "fast" residual spraying with alpha-cypermethrin (Fendona®) (FS) of dengue-positive and high-risk houses and space treatments with *d*-phenothrin (Anvil®) (ST); antilarval measures with insecticide (ALI), with CP and temephos (Abate®) pellets (TP) + ST; ALI + FS + ST; and the conventional approach to control, including larviciding with granular temephos, elimination of disposable containers, and ST with permethrin (Aqua Reslin® Super). The outcome of these interventions was evaluated by entomological assessments and passive dengue surveillance. After 6 months, integrated measures proved to be effective in reducing 85–98% of mosquito indicators, whereas no apparent suppression was observed where conventional measures (without ST) were undertaken. Community participation reduced larval indicators where no temephos was distributed.

Influence of the blood-meal source on the development of *Triatoma phyllosoma* (Hemiptera: Reduviidae)

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Triatoma phyllosoma is among the most important vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease, in Mexico because of its

occurrence in houses and high *T. cruzi* infection rates in villages of the Pacific Coast of Mexico. As with some other Triatominae species, *T. phyllosoma* usually feeds on mammals or birds, depending on the availability of these hosts. Most studies on *Triatoma* species (e.g., *T. infestans*, *T. brasiliensis*, *T. sordida*, and *T. pseudomaculata*) have shown that specimens fed on mammals (mice) have shorter developmental times and lower mortality rates than those fed on birds (pigeons). Taking into account previous information on the host preference of *T. phyllosoma*, the objective of this study was to determine the influence of the blood-meal source on the life cycle, feeding, and defecation patterns of *T. phyllosoma*. Specimens were separated in 2 groups of 150 1st instars, placed in plastic glasses (10 on each), fed weekly on New Zealand rabbits or leghorn hens, and checked daily to record molting and mortality. No significant ($P > 0.05$) differences were recorded in the life cycle among the specimens fed on hens (mean 192.6 ± 22.7 days) and those fed on rabbits (205.7 ± 34.8 days). Also, no significant ($P > 0.05$) differences were recorded among the specimens fed on hens and those fed on rabbits related to time for getting a complete blood meal, defecation patterns in most stages, and percent mortality of each stage. Our results showed that the nutritional characteristics of blood from hens were similar to those of blood from rabbits and different from those found in previous studies of unrelated species, but similar to results from studies on 2 closely related species, *T. longipennis* and *T. picturata*. Therefore, these results support the idea of an important association of *T. phyllosoma* with domestic birds, as cited in previous articles for related species.

Species diversity and biting behavior of sand-fly fauna in two foci of leishmaniasis in Mexico

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Leishmania mexicana is the protozoan causing cutaneous leishmaniasis in southern Mexico. Previous studies have reported 2 suspected vectors, *Lutzomyia olmeca olmeca* and *L. cruciata*. As part of the epidemiology of disease in Campeche, Mexico, we conducted an entomological survey in 2 allopatric foci in Campeche. We sought to document the fauna and biting habits of sand-fly species in the villages of Dos Naciones and La Guadalupe, Campeche, Mexico, during 1 transmission cycle. We found that both diversity and species richness were similar in Dos Naciones and La Guadalupe. However, when comparing Simpson's dominance

index, it was observed that La Guadalupe had a higher index than Dos Naciones. When comparing the biting rhythms of sand flies caught in Shannon traps, it was found that regardless of the location, sand flies were more active after dusk from 1800 to 2000 h. *Lutzomyia olmeca olmeca* was the only species caught in rodent-baited traps. Out of 16 species collected in both sites, transmission of the parasite appears to be restricted mainly to *L. olmeca olmeca* and secondly to *L. cruciata*. A brief discussion of the impact of deforestation on the sandfly fauna is presented in special reference to the suspected vectors.

A description of phlebotomine sand flies from the Peruvian Amazon

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Phlebotomine sand flies were collected from the Iquitos region of the Peruvian Amazon. Collections were made by utilizing CO₂-baited light traps suspended at different heights from the forest floor through the canopy (0, 1.5, and 8–10 m). A total of 443 sand flies were collected (35% males and 65% females). Eleven subgenera and 34 species were identified, including those in the genera *Psychodopygus*, *Nyssomyia* and *Trichophoromyia*, which represented the majority of the collections (22, 24, and 25%, respectively). The greatest diversity of species was found in the *Psychodopygus* (8), *Nyssomyia* (6), and *Aragoi* (4) groups. Species composition often varied based on the collection height, and included *Lutzomyia saltuosa* and *Lutzomyia* sp. 1 del Napo (sp. nov.) in the ground-level collections; *L. davisi* and *L. hirsuta* were most commonly collected at 1.5 m, as was the new species *Lutzomyia* sp. 2 del Napo; and *L. antunesi*, *L. yuilli*, and *L. umbratilis* were the predominant species in the collections from 8 to 10 m.

Genetic variability among populations of *Ochlerotatus taeniorhynchus* (Diptera: Culicidae) from Colombia

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Ochlerotatus taeniorhynchus (Wiedemann) is a zoophilic species and a vector of Venezuelan

equine encephalitis virus in Colombia and Venezuela. Its geographical distribution along the Atlantic and the Pacific coasts ranges from Massachusetts (USA) to Santa Catalina (Brazil) and from California to Peru, respectively. In Colombia, it is found in forests and inside houses along both coasts. Genetic diversity was compared among mangrove-forest populations of *Oc. taeniorhynchus* from 7 locations along the Atlantic coast in Colombia (San Bernardo del Viento, Coveñas, Cartagena, Barranquilla, Cienaga, Dibulla, and Riohacha) by examining genetic variation at 10 isoenzyme loci. The mean heterozygosity ranged from 32 to 45%, with 3.4 alleles detected per locus, and the percentage of polymorphic loci was 70%. Nei's genetic distances among populations ranged from 0.011 to 0.119. The estimated number of migrants (3.36) based on Wright's F statistic indicated low levels of gene flow among mangrove-forest populations of *Oc. taeniorhynchus* from the Atlantic coast from Colombia.

Genetic consequences of events on *Anopheles aquasalis* island populations

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Islands offer an ideal model to examine the consequences of historical and demographic events on evolutionary processes related to genetic variability. In mosquitoes, the degree of genetic differentiation is important for reconstructing phylogeographic histories and for clarifying features of population biology. Barbados, a young (700,000 BP) oceanic island, is set off from the Lesser Antilles chain. It was presumably colonized by mosquitoes via natural older invasions from the much older volcanic islands in the main Lesser Antillean arc, through recent human movement, or both. *Anopheles aquasalis* from the eastern coast of the Venezuelan mainland was used as representative of a large outbreeding continental population. Population samples were analyzed for genetic variability at 16 enzymatic loci. The degree of genetic differentiation was assessed between populations with respect to within-population indices of genetic diversity and Wright's F statistics. Analysis revealed differences in the average number of alleles per locus, heterozygosity, the proportion of polymorphic loci, and allele frequency distribution. However, the fixation index demonstrated little genetic structure between populations from Barbados and Venezuela. A tentative hypothesis for explaining these results is that the Barbadian population arose from bottlenecks of Venezuelan populations in steps through the Lesser Antilles.

Population genetics of *Aedes aegypti* in Oaxaca, Mexico

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Aedes aegypti is the primary urban vector of dengue and yellow fever viruses. Dengue fever and dengue hemorrhagic fever are public health problems in the tropics and subtropics, and are exacerbated by urbanization, population movement, and lifestyles that increase the man-made larval habitats of the mosquito vector. By using random amplified polymorphic DNA markers, we have shown that collections of *Ae. aegypti* from the southern Pacific Coast of México had as much genetic variation among collections 60 km apart as among all collections, and that mosquitoes from northeastern México were genetically isolated by distance and genetically homogeneous within a range of 90–250 km. We showed by variation in mitochondrial dehydrogenase subunit 4 gene (ND4) haplotypes that northeastern collections were genetically differentiated and had lower genetic diversity than Yucatan and western Pacific coastal collections. Because of the importance of this vector and the high frequency of dengue in Oaxaca, México, the aim of this study was to determine the genetic structure and vector competence of *Ae. aegypti*. We analyzed local patterns of gene flow in *Ae. aegypti* over a total distance of 300 km and among 4 populations in a city within a distance of 8 km. Genetic variation in ND4 haplotypes was examined in 60 mosquitoes per collection. Ten new haplotypes were detected and we are currently studying the vector competence of these populations. This research was supported by National Institutes of Health grant G-4632-1.

Mitochondrial DNA variation among *Aedes aegypti* populations in Venezuela

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We examined the distribution of mitochondrial DNA haplotypes among populations of *Aedes ae-*

gypti to test for genetic diversity by using an independent genetic marker. A 387-base-pair region of the reduced nicotinamide adenine dinucleotide dehydrogenase subunit 4 from the mitochondrial DNA (mtDNA) was amplified by the polymerase chain reaction (PCR) in 1,000 mosquitoes collected from 20 locations in different states of Venezuela. The PCR products were tested for variation by using single-strand conformation polymorphism analysis, and 8 haplotypes were detected. Examination of the results indicated that populations are not isolated by distance and that free gene flow occurs among the sites where these collections were made. However, it is clear that mosquitoes in the collection from Maracay (a city in Aragua State) are genetically distinct in mtDNA haplotype frequencies from mosquitoes in all other collections. Because the Maracay Health Service has implemented a strong insecticide-based vector control program during the last 6 years, this may provide conditions for selection of some haplotypes. It will be interesting to determine if these haplotypes are associated with insecticide resistant markers.

Effects of sublethal doses of temephos on a Cuban strain of *Aedes aegypti*

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Temephos has been used in Cuba continuously since 1981 to control larvae of *Aedes aegypti*. This insecticide has been applied in containers for domestic use at 1 ppm/liter. This dosage may be affected when cleaning the containers and because of operational mistakes. Sublethal concentrations of temephos (30% lethal concentration [LC₃₀], LC₅₀, and LC₇₀) were calculated using Probit-log analysis and were applied during 24 h to 4th instars of a strain of *Ae. aegypti* from Havana City, Cuba. Twenty-five adults of each sex of those that emerged from the treated larvae, as well as controls, were reared in 30 × 30 × 30-cm boxes in the laboratory and fed a 10% sugar solution and guinea pig blood. A container with water was placed in the cage for mosquito oviposition. This experiment was replicated 5 times. The applied concentrations (0.01089 mg/liter [LC₃₀], 0.01386 mg/liter [LC₅₀], and 0.01764 mg/liter [LC₇₀]) increased female longevity. Both the treated and control females had 5 gonotrophic cycles, although the treated ones showed statistically significant less fecundity and egg fertility than the controls. The treated mosquitoes showed less population growth than the controls, as expressed by the finite rate of increase and intrinsic rate of natural increase.

Larval resistance of three populations of *Aedes aegypti* to temephos in Trujillo State, Venezuela

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The insecticide resistance problem, along with the absence of a domestic hygiene program in Trujillo State, Venezuela, have contributed to the high prevalence of dengue. The most widely used organophosphate insecticides are temephos as a larvicide and fenitrothion and malathion as adulticides for control of *Aedes aegypti*. The objective of this study was to determine the level of resistance of 3 populations of *Ae. aegypti* from the localities Hatico (HT), Tres Esquinas (TE), and Paramito (PT) in the state of Trujillo to temephos. Fourth-stage larvae were assayed by using World Health Organization methodology. Resistance to temephos was found in all strains when compared with the susceptible Rockefeller strain. Ratios of resistance were estimated as 9.1 (HT), 9.6 (TE), and 13.3 (PT) for the median lethal concentration (LC_{50}) and 13.9, 12.7, and 12.7 for the LC_{95} , respectively. Examination of the results indicates that insecticide resistance in *Ae. aegypti* is a serious problem of a focal nature that should be treated independently.

Laboratory and field evaluation of *Macrocylops albidus* for control of larval *Aedes aegypti* in Cuba

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Predatory capacities of *Macrocylops albidus* on larvae of *Aedes aegypti* in different containers were evaluated. Copepods were collected in 3 Cuban provinces and cultured under laboratory conditions at $26 \pm 2^\circ\text{C}$ and a 12:12 h light:dark cycle. Petri dishes and plastic 7-liter containers in the laboratory and 200-liter drums in field trials were used for predation experiments. A single adult female cyclopoid, previously held for 24 h without food, was placed in each petri dish with 50 newly hatched larval *Ae. aegypti*. Surviving larvae were counted after 24 h. Plastic 7-liter containers with 5 liters of dechlorinated water and 3 g of leaf litter were used to test 3 combinations of predatory densities: 25, 50, and 100 *Ma. albidus* and 300 larval *Ae. aegypti*. Field trials were conducted in drums with 200 l of dechlorinated water and 5 g of leaf litter for 2 combinations of predatory densities: 100 and 200 *Ma. albidus*, each with 300 larval *Ae. aegypti*. All experiments were replicated 5 times and

separate controls with no copepods were studied in similar containers. Surviving larvae and remaining copepods were counted after 6 days. Analyses of variance showed a significant reduction of larvae in all the treatments with copepods.

Predatory capacity of *Piona amimitli* on larval *Culex restuans*

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Recognition of the importance of members of the *Culex pipiens* species complex as vectors of different diseases has been increasing during the last few years, mainly because they are involved in the transmission of West Nile virus. This motivated us to conduct a study on the predatory and searching capacities of the water mite *Piona amimitli* on larval *Culex restuans*, by examining the regression coefficient. We studied the mite's preference selection for the 1st 3 mosquito instars and found that the 1st instar was preferred by the mite. During the 2nd test, 5 nymphs, 5 males, and 5 females of the mite were exposed to 5 densities of larval *Cx. restuans*. After 3 replicates, the total number of larvae consumed after 24 h was recorded and data were analyzed by linear regression and by Hollings's and Rogers's functional response models. According to regression coefficients, $b = 0.983$ for nymphs, $b = 0.953$ for females, and $b = 0.999$ for males. Searching capacities were $a = 0.041$ for nymphs, $a = 0.041$ for females, and $a = 0.041$ for males, with Hollings's and Rogers's values of $a = 0.2852$ for nymphs, $a = 0.3109$ for females, and $a = 0.2821$ for males.

Field evaluation of Novaluron (OSCAR 100 CE) to control larvae of the malaria vector *Anopheles pseudopunctipennis* in Monterrey, Mexico

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Novaluron (OSCAR 100 CE) is an insect growth regulator with great potential as a supplement to temephos for larval control. Increased reports of te-

mephos resistance after decades of use against carriers of vector-borne diseases in endemic countries justify the need to evaluate new larvicide alternatives. Three dosages of Novaluron in water were tested in the field trial: 0.1, 0.2, and 0.3 ml/m³. Fifty larvae of the malaria vector *Anopheles pseudopunctipennis* were placed in 50 2-gal (7.6-liter) plastic trays. Trays were positioned in a riverbed and exposed to natural ambient conditions. To better compare Novaluron effectiveness, a commercial formulation of temephos also was tested. Each dosage was replicated in 10 trays, including controls. Mortality was checked after 24 h, 48 h, 72 h, and weekly for 7 wk. Examination of results showed higher and similar mortality for doses at 0.1 and 0.3 ml/m³. Temephos killed 100% of larvae at all time intervals up to 7 wk. Residuality of Novaluron was determined up to week 7. Novaluron may be a good choice for integrated larval control and when applied in rotation may delay larval resistance to temephos.

Field trials of Novaluron against *Anopheles albimanus* and *An. pseudopunctipennis* in southern Chiapas, Mexico

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Biorational agents, which are alternatives to synthetic chemical larvicides, are safer to humans, nontarget organisms, and wildlife. Among these, insect growth regulators are products that inhibit molting or synthesis of chitin. Novaluron, a novel chitin-synthesis inhibitor, recently has been evaluated against agricultural pests, but information about its effect against mosquitoes is limited. This project aimed to conduct World Health Organization Pesticide Evaluation Scheme phase II studies of Novaluron (Rimon 10 EC, Makhteshim, Israel) against the malaria vectors *Anopheles albimanus* and *An. pseudopunctipennis*. Studies were conducted during August–October 2003 by using 1 m² × 0.4-m sun-exposed plots. Resulting 99% lethal concentration (LC₉₉; X = 3.33 mg/liter), 2×, and 3× dosages were tested in triplicate. Three types of control plots were prepared: temephos (Abate and #61650; pellets 5%, Clarke, Roselle, IL) at 20 mg/liter; untreated plots where apparent fish predators of insects were manually eliminated; and untreated, undisturbed plots. Residual effect was evaluated by placing floating cages with 25 3rd- to 4th-stage larvae (2 per plot) and assessing natural larval population trends of *An. albimanus*. Through 5 wk, 100% mortality was observed with all concentrations of Novaluron and 100% reduction of adult emergence of natural populations has been ob-

served. Temephos has also produced the same effect.

Field trials of *Bacillus thuringiensis israelensis* polymer-based granular formulation with *Culex quinquefasciatus*

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A polymer-based granular formulation of *Bacillus thuringiensis israelensis* was evaluated for its efficacy against larval *Culex quinquefasciatus* in 45-m² permanent ponds in the Pesqueria River. Treatments included active ingredient (unformulated and formulated) and untreated controls. Larval abundance for several posttreatment periods was analyzed through 21 days. When larval abundance during sampling dates was analyzed, statistical differences were found among treatments after 21 days after treatment. The formulated active agreement was the best treatment. According to our results, the possibility of using polymer-based granular formulation over conventional mosquito control methods is now available.

Field evaluation of *Bacillus thuringiensis* subsp. *israelensis* polymer formulations in comparison with unformulated active ingredient

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A technical powder of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) was obtained by fermentation. After the *Bti* was evaluated against larval *Culex quinquefasciatus* collected from field, it was formulated with 2 natural polymers and evaluated against mosquito larvae in 200-liter plastic containers, in a random square design with 3 treatments and a control, applied at dosage of 13.7 mg/liter. Total larval population densities were recorded every 3–4 days after treatment by taking 10 dipper

samples from each treatment. Examination of the results showed significant larval reductions of *Aedes aegypti* and *Culex* sp. with 2 of the *Bti* polymer formulations compared with the control for more than 60 days after treatment. In contrast, a treatment with unformulated *Bti* powder showed toxic activity for only 3–6 days after treatment.

Biochemical characterization of insecticide resistance in a population of adult *Aedes aegypti* (L.) in the Baja California Peninsula, Mexico

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A discriminant dosage of permethrin active ingredient at 172 µg/ml was used to select individual *Aedes aegypti*. Mosquitoes were collected from 4 different municipalities located in northern and southern sectors of the Baja California Peninsula. Individuals were selected based on the presence of similar responses to insecticide. We exposed groups of 90 female *Ae. aegypti* to a discriminant dose, and after producing 50% mortality, individuals were divided into 2 categories: dead and survivors. Members of each of these groups were dissected to separate the head, thorax, and abdomen. Biochemical testing was performed on the head and thorax to determine enzyme activity, such as that of alpha and beta-esterases, glutathion-S-transferase, acetylcholinesterase, insensitive acetylcholinesterase, and mixed-function oxidases. Final results were compared with those from a susceptible New Orleans strain of *Ae. aegypti*. All populations that we studied consistently showed presence of alpha-esterases. Beta-esterases, the main resistance mechanism in permethrin-selected populations, although present in high proportions, did not reveal a clear pattern of its effect. Activity of glutathion-S-transferase appears to be the principal mechanism in the survivors tested.

Susceptibility of *Aedes aegypti* (L.) to insecticides in Nuevo Leon, northeastern Mexico

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Dengue control has become more difficult because of the development of resistance in the vector *Aedes aegypti* to the majority of the commonly used chemicals. This has occurred with different groups of insecticides (e.g., organochlorines, organophosphates, and pyrethroids). In the state of Nuevo León, Mexico, there is no information about the history of development of resistance of *Ae. aegypti* to the commonly used adulticides. The aim of this study was to investigate the status of susceptibility and resistance of *Ae. aegypti* to organophosphates, carbamates, and pyrethroids in 9 municipalities of Nuevo León. Adults were exposed to insecticide-impregnated papers for a period of 2 h, and mosquito mortality was evaluated every 5 min, with the final reading being taken at the end of 24 h. Probit analysis was used to generate median lethal time (LT₅₀) values. The insecticides used were 0.1% propoxur, 5% malathion, 0.75% permethrin, 0.05% deltamethrin, and 0.05% lambdacyhalothrin. Deltamethrin gave the lowest LT₅₀ value, ranging between 8.27 min (Escobedo) and 16.34 min (Villa de Santiago). On the other hand, propoxur produced the highest LT₅₀ values, ranging between 94.41 min (Linares) and 139.46 min (Villa de Santiago).

Assessment of damage caused by *Tapinoma melanocephalum* (Hymenoptera: Formicidae) on eggs of *Aedes aegypti* under laboratory conditions

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In Cuba, few studies have been conducted on *Tapinoma melanocephalum*, an ant that apparently damages cultivated sugar cane, citrus fruits, and coffee trees. The present study evaluates, for the 1st time, this ant's behavior in the insectary. Paper strips containing 100 eggs of *Aedes aegypti* were placed in 3 randomly selected sites along which ants passed (right floor, left floor, and on mosquito cages), with 3 replicates. Eggs belonged to 2 different oviposition times (i.e., those laid within the previous 24 h and those stored in the laboratory for 8 months). The latter group of eggs was collapsed and dehydrated. After 24 h, the egg-containing paper strips were collected and examined with a stereoscopic microscope and the eggs were counted. Data were analyzed and results confirmed that ants preyed on eggs from both groups but caused more damage to the recently laid eggs. Chi square and Kruskal-Wallis tests were conducted for the vari-

able "site" and its replicates. No significant differences were observed. A Z-test was calculated to compare proportions for the variable "age of eggs," which showed a marked preference for recently laid eggs regardless of the site where they had been placed.

Mosquitoes (Diptera: Culicidae) from Oaxaca, México

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From a medical point of view, mosquitoes are very important because several species take blood meals from vertebrates, including humans, and members of several genera (e.g., *Anopheles*, *Aedes*, and *Culex*) transmit diseases, including dengue, malaria, and encephalitis. Our objectives were to identify and prepare a checklist of the mosquitoes of Oaxaca State in southern Mexico. All identifications were made of larval mosquitoes that had been collected from July 1997 to July 2003 during the entomological surveillance program conducted by vector control personnel from the State Secretary of Health. Mosquito larvae were preserved in 70% ethyl alcohol and sent to the Oaxaca State Public Health Laboratory for identification. We identified 28 species in the following 10 genera: *Aedes* Meigen, *Ochlerotatus* Lynch-Arribalzaga, *Culex* Linnaeus, *Anopheles* Meigen, *Limatus* Theobald, *Uranotaenia* Lynch-Arribalzaga, *Psorophora* Robineau-Desvoidy, *Toxorhynchites* Theobald, *Haemagogus* Williston, and *Wyeomyia* Theobald. *Aedes aegypti*, *Ochlerotatus epactius*, and *Culex quinquefasciatus* were the species with the broadest distributions in Oaxaca.

Aquatic Diptera as a potential pathological risk in three recreation parks along the "La Silla" River in Guadalupe, Nuevo León, México

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Knowledge of aquatic Diptera in public parks gives several benefits. One of these is information about the entomological risk to public health caused by the Diptera that transmit diseases of humans. This work was focused on determining the diversity of Diptera and locating the possible breed-

ing sites of vectors in 3 recreational parks along the La Silla River in Guadalupe, Nuevo Leon, Mexico. Samples were collected every 15 days between May 22, 2002, and September 18, 2003. Samples were obtained by using a triangular net by introducing it into the river and removing 1 m² of substrate. The samples were placed in a plastic bag and preserved in 96% ethyl alcohol and transported to the laboratory for identification and counting. From 30 field collections, we obtained a total of 4,365 dipterans in 5 families (Chironomidae, Culicidae, Ceratopogonidae, Dolichopodidae, and Empididae) and 5 genera (*Beardius*, *Culex*, *Hemerodromia*, *Atrichopogon*, and *Glutops*). The highest density was that of *Beardius*, with 4,350 specimens collected. A single larvae of *Culex* was collected, which was the only species with a potential risk to humans as a vector of West Nile virus.

Predictive value of environmental variables and mosquito abundance over malaria morbidity in Bolivar State, southeastern Venezuela

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During 2000, 29,887 cases of malaria, which was considered an epidemic level for this disease, were reported in Venezuela. This phenomenon seemed to be linked to local climate changes, which principally increased temperature and affected rain patterns. The municipality of Sifontes in Bolivar State in southeastern Venezuela is considered a high-risk malaria area. To identify malaria-predictive environmental variables at the local level in a defined period of time, all-night human-landing catches were made in this area monthly from January 1999 to April 2000. Multiple regression analysis of monthly mosquito abundance plus environmental variables obtained at local stations indicated that the predictive variables of relative humidity ($P = 0.036$) and mosquito abundance ($P = 0.049$) had significant statistical effects on variability in the monthly parasite index (MPI). Likewise, the P -value suggested that rainfall ($P = 0.152$) and mean temperature ($P = 0.174$) variables did not have predictive value for the MPI in this area. Additionally, variance analysis ($F = 4.14$, $P = 0.031$) supports the regression analysis between the environmental variables and MPI. The importance of these climatic variables over the malaria morbidity is discussed. This study was supported by project VEN/96/021-023 and Inter-American Institute for Global Change Research (IAI-CRN).

Anophelines in the municipality of Villavicencio, Department of Meta, Orinoquia Region, Colombia

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Collections of adult and immature anophelines were made to study their behavior and habitat preferences at 11 locations in the municipality of Villavicencio, Department of Meta, Orinoquia Region, Colombia. Urban and rural areas were sampled from August to November 2002 and in April 2003. Adult mosquito collections were made between 1800 and 2100 h by using human bait, Shannon traps, animal bait, and resting sites and stored until isofamilies were obtained for their correct identification. In order of abundance, the species collected were *Anopheles marajoara*, *An. rangeli*, *An. braziliensis*, and *An. darlingi*. Biting rate peaks occurred in the peridomiciliary area for *An. marajoara* between 1900 and 2000 h and for *An. darlingi* between 1800 and 1900 h, whereas for *An. rangeli* and *An. braziliensis*, the peak of extradomiciliary biting activity occurred between 1800 and 1900 h. We characterized 44 breeding sites by taking into account the distance of the site to the nearest house, its physical and chemical aspects, its perimeter, and the species captured. These included fish ponds (65.9%), flooded grazing land (6.81%), and water drainage canals (6.81%). The remaining sites included streams, water springs, cisterns, excavations, ponds, small pools, and swamps with tropical palms ("moriche"). The immature species collected at these sites were the same as those collected as adults. The most abundant species was *An. marajoara*, which adapted better to breeding places characterized by total exposure to sunlight, by surrounding vegetation, and by neutral pH in clear or turbid water. *Anopheles marajoara* bites inside and outside human dwellings and was in much greater abundance compared with the presumed vector of malaria in this region, *An. darlingi*. This behavior is due to adaptations to land-use alterations, irregular control strategies, and invasion of its habitat by human immigrants because of civil unrest. Although this species has not been considered a malaria vector in Colombia, it has been found infected with human sporozoites of *Plasmodium* sp. For this reason, we think that *An. marajoara* and *An. darlingi* maintain malaria in this region, the 1st as an ancillary vector and *An. darlingi* as the main vector. Control strategies should be directed at developing primary prevention activities with the community

in order to clear the borders of fish ponds, as well as alternatives for personal protection.

Larvicidal activity of essential oils on *Aedes aegypti* (Diptera: Culicidae)

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Dengue and dengue hemorrhagic fever, the most important arboviral disease for humans in the world, are on the increase in most countries in the Americas. For many decades, the strategy for control of mosquito vectors was based on the use of chemicals; however, in recent years alternative control approaches that are more ecologically safe and sustainable have been sought. Essential oils derived from certain plants exhibit toxic effects on many insects, including mosquitoes. In this study, we evaluated the larvicidal activity of essential oils isolated from leaves of *Piper ossanum* and *Curcuma longa* against *Aedes aegypti*, the principal vector of dengue in the Americas. Three laboratory bioassays per essential oil were carried out by following standard World Health Organization methodology. Results were analyzed by probit-log software and demonstrated that these essential oils exhibited larvicidal activity against this mosquito. The median lethal concentrations (LC_{50} s) were 0.057 and 0.025%, and the LC_{95} s were 0.0076 and 0.0043% for *P. ossanum* and *C. longa*, respectively. The diagnostic concentration for each essential oil also was calculated. The results were compared with those obtained in previous studies of essential oils from *Eugenia melanadenia* and *Psidium rotundatum* against the same strain of *Ae. aegypti*. The possible implications and future perspectives for the use of essential oils against field populations are discussed.

Susceptibility of larval *Aedes aegypti* to insecticides in La Antigua, Veracruz, México

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The insecticide resistance problem along with the

absence of a domestic hygiene program in Veracruz State contributes to the high prevalence of dengue cases there. The objective of this study was to determine the level of susceptibility of *Aedes aegypti* from several municipalities to DDT, temephos, malathion, permethrin, cypermethrin, and biphenrin. We used World Health Organization (WHO) bioassay methodology. The results were analyzed by means of probit analyses by using WHO and maximum likelihood methods. Median lethal concentrations (LC₅₀) for DDT were 0.7044 and 0.7042 ppm for La Antigua and Cardel municipalities, respectively, and LC₉₅ values were 25.9938 and 26.7568 ppm for Salmoral and San Pancho municipalities, respectively. The LC₅₀ values for temephos were 0.003716 ppm for Cardel and 0.003773 ppm for Salmoral, whereas the LC₉₅ values were 0.018506 and 0.018383 ppm for Salmoral and San Pancho, respectively. The LC₅₀ values for malathion were 0.160403 and 0.171230 ppm for populations of *Ae. aegypti* from Cardel and San Pancho, respectively, and the LC₉₅ values for Cardel and San Pancho were 0.36114 and 0.429544 ppm, respectively. The LC₅₀ values for permethrin were 0.000044 ppm for Salmoral and San Pancho and 0.000045 ppm for Cardel. The LC₉₅ values were 0.0003 ppm for Cardel and 0.000306 ppm for La Antigua. The LC₅₀ for cypermethrin was 0.00002 ppm for La Antigua and Cardel and 0.00011 ppm for San Pancho, whereas the LC₉₅ was 0.000106 ppm for San Pancho and 0.000112 ppm for Salmoral. The LC₅₀ values for biphenrin were 0.000041 ppm for Cardel and 0.000044 ppm for Salmoral and San Pancho. The LC₉₅ values were 0.00021 and 0.000239 ppm for Cardel and Salmoral, respectively.

Preliminary observations on the susceptibility of *Lutzomyia evansi* to fenitrothion and lambda-cyhalothrin in Trujillo State, Venezuela

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Lutzomyia evansi is considered an alternative vector of visceral leishmaniasis in Colombia and Venezuela. In Trujillo State, Venezuela, a study on the bionomics of this species has revealed that it appears to be associated with the main focus of visceral leishmaniasis, with rates of natural infection of 0.23%. This species is anthropophilic with characteristic intra- and peridomestic behavior. By using this information, we studied the susceptibility of this species to selected insecticides under experimental conditions to establish the baseline susceptibility to fenitrothion and lambda-cyhalothrin. *Lutzomyia evansi* was collected in Pajones (9°30'28"N, 70°33'19"W), where no insecticides are used. The bioassay was carried out by residual exposure. The results revealed that these insecticides have a high

toxicity against this species; with a diagnostic concentration of 0.018% for fenitrothion and 0.0063% for lambda-cyhalothrin. The median lethal concentration (LC₅₀) and LC₉₅ were 0.012 and 0.05%, respectively, for fenitrothion, and were 0.0004 and 0.0017%, respectively, for lambda-cyhalothrin. These results are important because this is the 1st time that lethal concentrations have been determined for *L. evansi* and they can be used to establish comparisons with other populations of *L. evansi* originating from areas of high insecticidal pressure.

***Aedes aegypti* in Brazil: eradication really happens?**

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The mosquito *Aedes aegypti* was introduced into Brazil for the 1st time during the 17th and 18th centuries with the slave traffic from Africa. In the 1950s, there was an eradication campaign in South America and *Ae. aegypti* was considered eradicated in many countries, with Brazil among them, but in 1959, the species was reported to be present in Brazil again. We analyzed data on 153 American mosquitoes sequenced for a 361-base-pair fragment from the mtDNA *NADH4* gene. Molecular variance and nested clade analysis indicated the presence of 2 major groups, separated by 8 mutational steps (95% confidence limit). One of these groups (group 1) is related with Senegal populations (Africa), whereas the other group (group 2) is related to other African, Asiatic, and American populations. The analysis showed that group 2 was 2-fold more polymorphic ($H = 0.8131$) than group 1 ($H = 0.4033$) and from analysis of variance, it was found that 72% of the variability occurs between these 2 groups. Examination of these data suggests that there were at least 2 introductions of *Ae. aegypti* into South America, with the former from Senegal that was supposed to be eradicated and a later one following the Africa-Asia-North America route.

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