

## MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—AN ELEVENTH SYMPOSIUM

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**ABSTRACT.** The 11th annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 67th Annual Meeting in Dallas, TX, in February 2001. The principal objective, as for the previous 10 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 45 presentations that were given orally in Spanish or presented as posters by participants from 8 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*), malaria (*Anopheles albimanus* and *Anopheles aquasalis*), leishmaniasis (*Lutzomyia*), murine typhus, and Chagas' disease (*Triatoma*). Related topics included biology and control of *Rhodnius*, scorpions, *Loxosceles* spp., *Chironomus plumosus*, and *Musca domestica*.

**KEY WORDS** Mosquitoes, mosquito control, *Aedes*, *Anopheles*, *Culex*, *Lutzomyia*, *Triatoma*, scorpions, *Loxosceles*, *Musca domestica*, *Chironomus*, resistance

### 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 at the AMCA Annual Meeting in 1991 and symposia have been held at subsequent meetings. In addition to providing a forum for scientists whose primary language is Spanish, the session promoted interaction and esprit de corps within this group; interaction with 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 45 presentations that were given in Spanish by participants from 8 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 scorpions, *Loxosceles* spp., *Chironomus* spp., and *Musca domestica*, and vectors of dengue (*Aedes aegypti* (L.)), malaria (*Anopheles albimanus* Wiedemann and *Anopheles aquasalis* Curry), leishmaniasis (*Lutzomyia*), and Chagas' disease (*Triatoma*). Summaries of 9 previous symposia have been published (Clark and Suarez 1991, 1992,

1993; Clark 1995, 1996; Clark and Rangel 1997, 1998, 1999; Clark et al. 2000).

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### PRESENTATIONS

#### Vector populations (order: Diptera) in the Panama Canal areas (1999–2000)

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The Panama Canal Authority maintains a surveillance program of mosquito and sand fly populations within its operational areas. The need for this program is to protect its clients, visitors, and the workforce against vector-borne diseases. For this purpose, 19 New Jersey light traps are located in different areas, supplemented by the active search for mosquito larvae along the 50 mi of property along the waterway. Collections were identified daily and results were analyzed weekly. Results obtained during the wet and dry seasons are presented. Major vectors of diseases are in the genera *Anopheles*, *Culex*, *Aedes*, *Mansonia*, and *Lutzomyia*. The most important mosquito vectors are

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*Anopheles albimanus* and *Aedes aegypti*. Monitoring insect vector populations is a very important step before implementing routine control operations, which are structured on an integrated pest management system that always attempts to protect the environment. The program is based on the elimination or control of breeding sites and a continued monitoring of the incidence of disease within the Panama Canal areas.

#### **Entomological surveillance of *Aedes aegypti* and *Ae. albopictus* ovitrapping program in Veracruz, México, during 1998–1999**

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From 272 mosquito samples received from the state of Veracruz, 3 samples from Martínez de la Torre had *Aedes albopictus*. This is the 1st record of this species in the state. The entomological surveillance program was enhanced and included the collection of mosquito larvae from artificial containers and the use of ovitraps in outdoor and indoor urban areas to detect *Ae. albopictus* populations in selected communities throughout the state. Eggs from ovipaddles were hatched and reared with dog food powder to obtain 4th-stage larvae or adults for species identification. A total of 5,424 ovipaddles from 10 sanitary districts was received at the laboratory during 2 years. In 1998, 1,743 samples (62%) had *Aedes aegypti* and 1,087 (38%) were negative. During 1999, 1,884 (73%) ovipaddles were positive for *Ae. aegypti*, 696 (27%) were negative, and 2 were positive for *Ae. albopictus* and *Ae. aegypti* in 1 district. This finding confirms the record of *Ae. albopictus* in México and suggests the species is spreading toward southern México.

#### **Rapid spray of insecticide in high-risk houses as an alternative for the control of *Aedes aegypti***

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We evaluated the rapid spray of insecticide for the control of *Aedes aegypti* in 6 cities in southern

México. Treatments were insecticide application of all the houses within a city; focal treatment of high-risk houses, classified by the premise condition index (PCI); and larval control with conventional larviciding (temephos). The PCI was based on the good, moderate, or bad appearance of house, shade, and tidiness of backyard. Insecticide droplets of 50–100  $\mu\text{m}$  were applied with a mistblower at rates of 13 and 20 mg active ingredient/m<sup>2</sup> for deltamethrin and bifenthrin, respectively. Insecticide impact was measured on larval and adult aedine indicators, whereas insecticide residuality effect was estimated. Each house was treated in 10 min. Insecticide residual effect with 75% mortality lasted for up to 19 wk. All aedine indices were reduced in insecticide-treated houses as compared to control areas, and adult populations were suppressed from premises for up to 12 wk. These reductions were more apparent in the full treatment areas, but not different than in focally treated cities. Therefore, the full or focal rapid spray to control *Ae. aegypti* proved more effective than conventional larviciding. These strategies remain to be evaluated on whether 1 or the other reduces or suppresses dengue transmission.

#### **Laboratory evaluation of permethrin-impregnated bed-nets against *Aedes aegypti***

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Impregnated bed-nets have been an important tool to prevent mosquito-biting activity. The efficacy of permethrin on impregnated nets of 100% nylon (1-mm mesh), 100% nylon (0.1-mm mesh), and 100% polyester (2-mm mesh) was evaluated against *Aedes aegypti* under laboratory conditions. All 3 types of bed-net material were impregnated with 1, 0.5, 0.25, 0.15, 0.1, 0.05, and 0.01% permethrin. The knockdown time and knockdown and mortality rates were registered weekly for 2 months after impregnation. The test material impregnated with 1, 0.5, and 0.25% permethrin caused 100% knockdown and 100% mortality rates during the 2 months after impregnation and the average time required for complete knockdown was between 2.25 and 4.50 min. Mortality registered with permethrin concentrations of 0.15 and 0.1% were 85–100% and 60–95% during the 2 months after treatment, respectively. Knockdown time was between 5.75 and 10.0 min during the 1st and 8th wk after impregnation, respectively. No significant difference in mortality rates was found among the 3 materials tested.

### Chlorine bleach as an ovicide for *Aedes aegypti* eggs

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Chlorine bleach (5.25% sodium hypochlorite solution) was studied as an ovicide for *Aedes aegypti*. Strips of paper towels with 100 or 200 eggs were set on the bottom or the side of 6-liter plastic containers. Pure or 50% bleach was added with a syringe to the eggs and after 5, 10, 15, 20, 30, 45, or 60 min the recipient was filled with tap water covering the eggs. Each test was replicated 3 times with controls (recipient with eggs and tap water). Larvae were counted 1 wk after the onset of trials. The longer the time of exposure to the bleach the fewer larvae were counted after 1 wk in the experimental containers. Exposure to pure chloride bleach for 15 min produced an average of 4.67 larvae and a similar exposure to 50% bleach for the same time produced 64.53 larvae versus a mean of 105.0 in the controls ( $P < 0.05$ ). Trials with six 55-gal metal drums, which are widely used in the Dominican Republic for water storage, were divided in half by plastic walls. One half was used as a control and the other was treated with pure bleach for 15 min. A mean of 3.3 larvae was found in the bleached half versus a mean of 58 larvae, pupae, or both in the controls ( $P < 0.05$ ). Utilizing 8 metal drums full of eggs, 3 were treated with pure bleach that was applied with a sponge to soak the inner walls and 15 min later filled with tap water. Five controls were used. Our results showed that the experimental drums had 1.0 and 3 larvae, whereas the controls had hundreds of larvae.

### Larvicidal activity of extracts of four species of *Agave* on *Aedes aegypti* larvae

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The toxic impacts of natural products obtained from algae and plants on insect larvae were studied as a possible strategy for dengue vector control in México. In 1999, México experienced 14,655 cases of classical dengue and 25 cases of dengue hemorrhagic fever; 63.6% of these cases were reported in the northern states of Nuevo León and Tamaulipas. These states have many species of plants in the family Agavaceae, which has shown different

biological effects on bacteria, fungi, insects, animals, and humans. The purpose of this study was to evaluate the larvicidal impact of extracts obtained with acetone and methanol of *Agave americana*, *A. lechuguilla*, *A. lophanta*, and *A. scabra*. The greatest toxicity was found at 100 ppm in the methanolic extract of *A. lophanta* (67% mortality) and *A. scabra* (>50%). The extracts obtained with acetone at 100 ppm from *A. americana* and *A. lechuguilla* registered low larvicidal effects (<35%). The active extracts were tested with colorimetric chemical reactions to identify functional groups and secondary metabolites (possible toxic compounds). Both extracts (methanol and acetone) were positive to esters, terpenes, and saponins, whereas coumarins were positive only for the methanolic extracts.

### Why doesn't dengue exist in Ponciano Arriaga, México?

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Although several cases of dengue were registered in the Huasteca Potosina in 1998, no case was recorded in the small town of Ponciano Arriaga. Because of that, an entomological survey was undertaken to better understand why dengue was not found there. We found that people from Ponciano Arriaga store water in cement containers that have been closed with materials such as cement, metallic covers, or plastic films. In the open containers, we found 2 notonectid species, *Notonecta irrorata* and *Buenoa scimitra*, and although the containers were cleaned monthly, the predators apparently persisted. Accordingly, community members and health ministry personnel gave information about *Aedes aegypti*, dengue fever, and vector control without insecticides.

### Murine typhus in the state of Veracruz, México

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Between 1990 and 1996, 667 cases of rickettsiosis (86% of which were murine typhus), were re-

corded in México. Previous observations indicated that this disease had a higher incidence in females older than 25 years of age. In 1997, a case of murine typhus was identified in Zaragoza, a small community (8,000 inhabitants) in the state of Veracruz. A total of 130 serum samples from patients with clinical symptoms associated with this disease were sent to INDRE. Indirect immunofluorescence (IIF) for immunoglobulin M was positive in 106 samples (25 males and 81 females). One of 3 rats collected in Zaragoza was highly positive by IIF in liver and kidney extracts and the other 2 were low positives. Twenty-two ectoparasites were obtained from the rats, including the natural vector of *Rickettsia typhi*, *Xenopsylla cheopis*. These results indicate that an outbreak of murine typhus occurred in 1997 in Zaragoza, Veracruz. As a control measure, rats were almost completely eliminated from the community by baited traps.

#### **Egg production, fertility, and defecation patterns of four Mexican Triatominae (Hemiptera: Reduviidae) species under laboratory conditions**

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In México, *Triatoma pallidipennis*, *T. longipennis*, *T. picturata*, and *T. phyllosoma* are considered to be the most important vectors of Chagas' disease. Studies of their biology can increase the understanding of their role in *Trypanosoma cruzi* transmission to humans and could improve control programs. For these reasons, research on mean egg production, fertility of eggs, and defecation patterns was conducted in these 4 species. The species have been maintained under laboratory conditions at 27 ± 2°C and 55 ± 5% relative humidity, and fed on New Zealand white rabbit weekly. During a study period of 90 days, a mean of 2.0 eggs/female/day and egg fertility of 98.8% ( $n = 1,757$ ) were recorded for *T. pallidipennis*, 2.2 eggs/female/day and egg fertility of 78.3% ( $n = 1,953$ ) were recorded for *T. longipennis*, 2.01 eggs/female/day and fertility of 65.1% ( $n = 1,853$ ) were recorded for *T. picturata*, and 1.8 eggs/female/day and fertility of 84.2% ( $n = 1,597$ ) were recorded for *T. phyllosoma*. Most 1st-stage bugs (70–80%) defecated 1 min after feeding. These baseline data suggest that these 4 species can be considered as important vectors of *T. cruzi* to humans.

#### **Triatome bugs infected with *Trypanosoma cruzi* from dwellings in México**

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Chagas' disease is an important public health problem in Latin America. Triatomine bugs are natural vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Vector transmission is the main source of infection to humans. In the present study, the frequency of infection and the distribution of triatomine bugs was obtained to identify risk areas. During 1999 and 2000, we received 946 triatomine bugs collected from dwellings in 9 states in México. The frequency of infection was determined by searching for *T. cruzi* in the bugs' feces. Three *Triatoma picturata* collected in Nayarit were positive. The *Triatoma pallidipennis* collected in Morelos were also positive, as were 48% of those received from Michoacan. Positivity of *Triatoma dimidiata* was 26% in Hidalgo, 15% in Veracruz, and zero in Puebla. Ten percent of *Triatoma* sp. were positive and the remaining species were negative. Collection sites with triatomine bugs identified in Veracruz State included bedrooms, beds, and walls. We found that *T. pallidipennis* had the highest percentage of *T. cruzi*, followed by *T. dimidiata*.

#### **Association of scorpion sting cases with highly toxic scorpion species in México**

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Scorpions may sting any human but most frequently sting children and older people. After evaluating annual scorpion sting reports, 16 states located on the Pacific coast and inland are considered endemic. The purpose of this study was to associate the frequency of scorpion stings with the occurrence of highly toxic species. For this, taxonomic identification of scorpions was performed and compared to the distribution and number of scorpion stings in 10 endemic states. Between 1995 and 2000, we received 855 scorpions specimens from 17 states. The following data were obtained: Nayarit (4 species), Oaxaca (3), México (2), Puebla (2), Guerrero (2), Sinaloa (2), Queretaro (1), Colima (1), Durango (1), and Guanajuato (1). Of these states, Guerrero, Guanajuato, Colima, and Puebla have had more than 50,000 cases in the last 5 years. In Nayarit, although 5 species were found and 1 of them (*Centruroides noxius*) is the most toxic, the

number of stings reported was low compared to Guerrero, where 2 species were found. Apparently, no correlation exists between the number or variety of toxic scorpion species found and the number of sting cases reported by the state.

### Mortality by sting intoxication of scorpions in Guerrero, México (1998–2000)

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During 1998, scorpion sting intoxication was the 15th greatest cause of morbidity in México. Guerrero is one of the 16 states with highly toxic scorpions, including many important species and subspecies in the genus *Centruroides*. The objective of this project was to establish risk areas by studying morbidity and mortality. We conducted a transverse study to determine deaths by sting, cases, and distribution of death by scorpion species, through the analysis of death certificates and morbidity and population data, and by collection of scorpions. The study was performed in 6 of Guerrero's 7 sanitary districts. The highest morbidity rate was in the 2nd district, with 86.5 cases per 10,000 inhabitants. The 3rd district had a mortality rate of 1.8 deaths per 10,000 inhabitants and the 4th district had a lethality rate of 2.9 deaths per 1,000 cases registered. This district has the county with the highest morbidity rate (Olinalá with 477 cases), mortality rate (Copanotayac with 15 stinging deaths), and lethality (Zapotitlán Tablas with 333 deaths by sting). *Centruroides limpidus* was the most abundant species. Mortality and lethality did not share the same hyperendemic zones.

### Distribution of *Loxoscelidae* (Araneae: Araneomorphae) in Nuevo León, México

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The family *Loxoscelidae* includes the genus *Loxosceles*, which has about 90 species in the Americas. In the USA and northern México, *Loxosceles* *prisoner*, *L. arizonica*, and *L. deserts* have been reported, whereas in South America, *L. laeta*, *L. spadicea*, *L. rufescens*, and *L. gaucho* have been reported and found to cause severe cutaneous necrosis in man. All species in this genus have been reported to produce "loxoscelism." The objectives

of our study were to conduct studies to characterize and inventory the species the *Loxosceles* in Nuevo León, México; to provide elements to assist in the identification of *Loxosceles* species; and to gather data on their distribution and biology. A total of 204 specimens belonging to the Arachnological Collection were reviewed. Of these, 44% were males, 36% were females, and the remaining 20% were juveniles. We confirmed the presence of *L. devia* and *L. candela* in Nuevo León, as well as the 1st record of *L. belli*. According to the collection records, *L. devia* and *L. candle* are present in urban habitats and they are commonly found on the floor, and hidden between or under stones, or in cracks.

### Malaria vectorial capacity of *Anopheles punctimacula* in southern Chiapas, México

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We studied the bionomics of *Anopheles punctimacula*, an abundant species that readily bites humans, along the coastal plain of Chiapas, México. This mosquito has been reported elsewhere as a secondary malaria vector. Human landing collections were conducted to assess its abundance and biting cycle. Blood-engorged mosquitoes from indoor and outdoor resting collections were assayed to establish the human blood index. Daily changes in the parous-nulliparous ratios in *An. punctimacula* females were used to calculate daily survivorship and gonotrophic cycle and the vectorial capacity was calculated. The resulting human biting rate was 18.65 (4.53 mosquitoes/man/night). The biting cycle showed an early evening peak by *An. punctimacula*, with 50% of the bites occurring before 2200 h. The daily survival rate of *An. punctimacula* was 0.68 and a 4-day gonotrophic cycle was found ( $r = 0.709$ ;  $P = 0.0067$ ). The sporogonic cycle was calculated based on a mean temperature of 26.2°C, and was 8.97 days for *Plasmodium vivax* and 10.88 days for *P. falciparum*. Resulting vectorial capacity was 1.08 and 0.49, respectively, for *P. vivax* and *P. falciparum*. This figure was higher than those reported for other confirmed anopheline vectors in the area.

### Colonization of *Anopheles darlingi* from the Lacandon Forest, Chiapas, México

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*Anopheles darlingi*, the main vector of malaria in the Americas, has a limited distribution in Méx-

ico, although it has not been found to be infected with *Plasmodium*. The low population density of *An. darlingi* in the field and the difficulty in establishing laboratory colonies have contributed to a limited knowledge of this species. In March 2000, we collected *An. darlingi* on animals and humans in the Lacandón Forest in Chiapas State. Eggs obtained from 5 females through induced oviposition were taken to an insectary situated 280 km to the southwest. Larvae were reared and mating was induced in 270 F<sub>1</sub> adults with 12:12 h light:dark photoperiod, 30°C:25°C day:night thermoperiod, and aided from 1930 to 2030 h with a flashlight horizontally set at 0.15 m from the edge of a standard cage (0.6 × 0.6 × 0.6 m). Females were allowed to oviposit, and eggs were incubated for 48–120 h and had a 45% hatching rate. The F<sub>2</sub> larvae were fed with mouse feed. Larval survivorship was 98%, with mean pupation time of 11 days. Adults were offered blood meals every 48 h and female longevity averaged 30 days. Insemination in females ranged from 25% (F<sub>2</sub>) through 36% (F<sub>6</sub>). The colony has been maintained for 7 generations, with a production of 25 larvae in the F<sub>2</sub> generation to 510 in the F<sub>6</sub> generation.

### Phase III trials of bifenthrin to control *Anopheles* in southern México

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Although use of DDT in México must be discontinued by 2007, it has already been replaced by deltamethrin for malaria vector control. However, considering a rotation scheme for the long term is necessary to define which product(s), including new insecticides, can be used. Desired attributes of new insecticides are high killing and low repellent effect against mosquitoes; sustained residual effect on walls; low toxicity and persistence in the environment, wildlife, and humans; and acceptance by residents and spraymen. In this study, we conducted phase III studies (village-scale) to compare the efficacy of the indoor residual spray of the novel pyrethroid bifenthrin (OMS 3024) with deltamethrin against the malaria vectors *Anopheles albimanus* and *An. pseudopunctipennis*. Studies included baseline anopheline susceptibility against bifenthrin, and village-scale trials in 2 epidemiological situations where *An. albimanus* or *An. pseudopunctipennis* were the prevalent vectors, to establish the impact of bifenthrin on the mosquito populations and the appropriate frequency of insecticide application. Methods of evaluation included insecticide perfor-

mance, entomological impact, and acceptance of treatments by residents and spraymen. Overall results confirmed the potential of bifenthrin as a residual insecticide for the control of both malaria vectors in southern México.

### Morphological differences among *Anopheles vestipennis* populations

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Evidence for the existence of a species complex within *Anopheles vestipennis* is coming from many sources, including isozyme and random amplified polymorphic DNA polymorphism, egg ultrastructure, and ecology. In this study, we compared the wing length and sector dark spot (SD) length of sympatric anthropophilic and zoophilic *An. vestipennis* populations. The F<sub>1</sub> mosquitoes from females collected on human or animal hosts in Chiapas, México, were released in the center of an experimental hut. Side compartments contained 2 humans and an animal host. Faithful mosquitoes, that is, those selecting the same host from which parental females were collected, were recorded. The wing and SD lengths of anthropophilic mosquitoes (4,001.38 ± 18.73 µm, range 3,571–4,333 µm; and 703.36 ± 8.17 µm, range 547–857 µm, respectively) were significantly greater than those of zoophilic mosquitoes (3,919.47 ± 18.19 µm, range 3,428–4,285 µm, [ $t = 49.87$ ,  $df = 137$ ,  $P < 0.05$ ], and 676.81 ± 7.67 µm, range 571–809 µm, respectively, [ $t = 30.17$ ,  $df = 119$ ,  $P < 0.05$ ]). The ratio of wing length to SD length was lower in anthropophilic mosquitoes (5.7170 ± 0.16, range 4.84–6.78) compared with zoophilic mosquitoes (5.7238 ± 4.94–6.83;  $t = 14.06$ ,  $df = 93$ ,  $P < 0.05$ ). These results suggest segregation among populations with different feeding preferences.

### Morphometric variation in Colombian populations of *Anopheles darlingi*

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In the present study morphometric variation was evaluated to determine heterogeneity and to identify characters that have a genetically detectable component in a population of *Anopheles darlingi* from Colombia. The morphometric variation of the

costal patterns and hind tarsus II in eastern and western subpopulations was analyzed. Three hundred ninety-two offspring (257 females and 135 males) and 249 females from the Museum of Entomology of the Universidad del Valle were included in the analysis. According to the variation of the costal patterns, it was possible to detect up to 15 different types. The highest frequency was recorded in only 2 patterns, which actually were defined for all costal areas (=type I) and the junction of PSD+SP+PRSD (=type II). Type I was predominant in the eastern subpopulation, whereas type II was predominant in the western subpopulation. The sex as well as the costal patterns (I, II) affected the variation in some of the analyzed characters; an analysis of variance with the data of the offspring showed that only some characters of the costal spot (PHD, HP, PSP, SP) but they did not present sexual dimorphism. Independently of the costal pattern and of sex, of the 16 characters analyzed, only 3 were consistent with the differentiation of subpopulations, and this was sustained in a genetic component. Of the total variation of the phenotype, between 54 and 62% of the variation of the character (appraising dark area of the TIII-2/longitude of the TIII-2) was due to significant differences among the subpopulations. Other costal characters are population-specific depending on the analyzed pattern and the sex.

#### **Population genetics of *Anopheles albimanus* using microsatellite markers**

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In a previous study, we examined the distribution of mitochondrial DNA haplotypes among populations of *Anopheles albimanus* from Guatemala to test for gene flow barriers using a ~350-base pair sequence of the mitochondrial reduced nicotinamide adenine dinucleotide dehydrogenase subunit 5 (ND5) gene. Little variation was found among samples of populations or regions across 3 seasons. We have developed microsatellite markers to further analyze the population structure of *An. albimanus* with an independent marker. Three microsatellite primers (1-90, 2-14, and 6-41) produced consistent polymerase chain reaction products and presented 18, 20, and 12 alleles, respectively. Populations from different regions of Guatemala (northern, southern, and eastern) presented variations in the frequency of alleles for the 2 microsatellite markers analyzed. Comparison between the 3 regions using analysis of variance showed that 92.85% of variation in allele frequency arose among individuals in a collection, whereas only

4.23% of variation arose among collections in a region, and 2.92% of variation arose among regions. This analysis indicates that small variation exists among populations from northern, southern, and eastern Guatemala; this variation is higher than that detected with mitochondrial markers (1.67% variation among regions), but is smaller than that detected with intergenic spacers of nuclear ribosomal DNA (8.7% variation among regions).

#### **A population genetics study of *Anopheles darlingi* from Colombia based on random amplified polymorphic DNA-polymerase chain reaction and amplified fragment length polymorphism (AFLP) molecular markers**

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A molecular genetic study of 3 Colombian *Anopheles darlingi* subpopulations (2 western and 1 eastern) was performed with random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and amplified fragment length polymorphism (AFLP) molecular markers to clarify the taxonomic status of this species in Colombia. In this study, 46 polymorphic RAPD-PCR fragments produced by 6 primers were obtained for 64 DNA samples. A total of 202 AFLP polymorphic fragments from the 2 primer combinations was obtained for 71 DNA samples. Cluster analysis using a distance matrix with Phylip 3.5C and multiple correspondence analysis were used. For RAPD-PCR fragments, the consensus tree and multiple correspondence results showed significantly low evidence of geographical separation between eastern and western subpopulations; nevertheless the Fst data showed a high genetic flow between the 2 Colombian western subpopulations (Fst = 0.008). A clear geographic partitioning was obtained with the AFLP fragments. The geographical isolation between the eastern Colombian subpopulation and the 2 western Colombian subpopulations was better adjusted with Fst data obtained from the AFLP fragments (Fst = 0.213 and 0.220) than the Fst data obtained from RAPD fragments. These results showed evidence of a high genetic flow between the 2 western Colombian subpopulations (Fst = 0.096).

### Random amplified polymorphic DNA analysis of Colombian *Anopheles nuneztovari* populations

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Random amplified polymorphic DNA (RAPD) markers were used to analyze 119 samples of 3 Colombian *Anopheles nuneztovari* subpopulations to establish the possible existence of cryptic or complex species. Of the 554 RAPD primers screened, 9 produced a total of 65 polymorphic fragments ranging in size from 478 to 1,778 base pairs. A RAPD PLOT program analysis showed small genetic distances between the 3 subpopulations and did not allow a clear separation, using a 1-S distance matrix. Nevertheless, the genetic flow was significantly different. It was higher between western Colombian subpopulations (Lynch and Milligan  $F_{st} = 0.035$ ;  $N_m = 6.8$ ) and smaller between those and the eastern Colombian subpopulation ( $F_{st} = 0.08$ ;  $N_m = 2.8$ ). A distance dendrogram of the 65 fragments, using a Nei's unbiased genetic distance analysis, showed evidence that the 2 western subpopulations clustered separately from the eastern subpopulation in a consensus tree derived from the 100 unweighted pair-group method using arithmetic averages (UPGMA) trees. A multiple correspondence analysis generated 16 clusters and also depicted the geographical separation between the 2 western subpopulations and the eastern subpopulation. These results indicate that the subpopulations of Colombian *An. nuneztovari* are conspecific.

### Enzyme polymorphism among *Triatoma picturata* (Hemiptera: Reduviidae) populations from western México

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Isozyme variability in populations of the Chagas' disease vector *Triatoma picturata* was determined

by means of starch gel electrophoresis. Samples were taken from 5 localities across the range of this species in western México, where this species is one of the most important vectors. Zymograms for proteins coded by a total of 4 loci of 3 enzymes (esterases [Es EC 3. 1. 1. 1], malate dehydrogenase [MDH EC 1.1.1. 37], and malic enzyme [Me EC 1.1.1.40]) were obtained. Proportion of polymorphism loci, endogamy, mean heterozygosity per locus, gene flow, and Nei's genetic distance among populations were calculated. Only 1 (33%) locus was polymorphic; endogamy was 1, heterozygosity was 0. Gene flow was 0.9376 and Nei's genetic distances were minimal (0–0.002) among populations. According to the data above, all of the subpopulations of *T. picturata* that were studied could be considered a single widely distributed population.

### Oviposition preference of *Anopheles albimanus* for 5 plant species under laboratory conditions

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We studied whether the attraction that gravid *Anopheles albimanus* females have for water containing aquatic plants (*Brachiaria mutica*, *Cynodon dactylon*, *Jouvea straminea*, *Fimbristylis spadicea*, or *Ceratophyllum demersum*) is related to chemicals (released substances from plants, water, or both) or physical stimuli (shape or color). Gravid females were offered water bowls containing natural plants (NP) in larval habitat water (LW) as oviposition substrates, or artificial plants (AP) in distilled water (DW); and NP in DW, or AP in LW as oviposition substrates. Gravid *An. albimanus* females deposited more eggs in bowls containing NP in LW than in bowls containing AP in DW. Also, gravid mosquitoes deposited more eggs in bowls containing NP in DW than in bowls containing AP in LW. These results were confirmed in experiments conducted in an olfactometer, indicating that female response was mediated by chemical cues from plants. Gas chromatography and mass spectrophotometry analysis of the organic extracts from all 5 plants showed a mixture of terpenoid and alcohol compounds, including guaiacol, phenol, isoeugenol, longifolene, caryophyllene, phenyl ethyl alcohol, and *p*-cresol. These results suggest that middle-range volatiles from plants function as chemical



cues for the female *An. albimanus* oviposition response.

**Advances in the characterization of insecticide resistance of the strain pattern "Chagas" of *Rhodnius prolixus* (Hemiptera: Reduviidae) in Venezuela**

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In the detection of resistance to insecticides, any study should begin by evaluating a sensitive laboratory strain. In the case of the triatomines, a sensitive strain must have more than 5 generations in the laboratory without the addition of field material. To determine the baseline of resistance to different insecticides, a laboratory (S) strain of *Rhodnius prolixus* was tested with organochlorine, organophosphate, carbamate, and pyrethroid insecticides. Topical applications of acetone solutions of insecticide were made to the dorsal abdomen of 1st (0.1  $\mu$ l) and 5th nymphal instars (0.5  $\mu$ l). Results showed, for 1st-stage nymphs, median lethal doses ( $LD_{50s}$ ) of dieldrin, 243 ng/insect (i); fenitrothion, 2 ng/i; methyl pyrimiphos, 13 ng/i; propoxur, 13 ng/i; lambdacyhalothrin,  $1.4 \times 10^{-3}$  ng/i; deltamethrin,  $5 \times 10^{-4}$  ng/i; and cyfluthrin,  $6 \times 10^{-2}$  g/i. In nymphs, the  $LD_{50s}$  were dieldrin, 2,460 ng/i; fenitrothion, 100 ng/i; methyl pyrimiphos, 714 ng/i; propoxur, 690 ng/i; lambdacyhalothrin, 6.6 ng/i; deltamethrin, 18 ng/i; and cyfluthrin, 380 ng/i.

**Resistance of *Aedes aegypti* to pyrethroids in municipalities of the Aragua State, Venezuela**

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In 3 strains of *Aedes aegypti* from the municipalities of Mario Briceño Iragorri (MBI), José Félix Rivas (JFR), and Girardot of Aragua State (1981), we studied the resistance of 4th-stage larvae to the pyrethroids cyfluthrin, lambdacyhalothrin, and deltamethrin, in comparison with the susceptible Rockefeller strain using the World Health Organization method. Resistance to lambdacyhalothrin was found in all strains; the MBI and Girardot strains showed resistance to cyfluthrin and to deltamethrin, respectively. Evaluating the synergist, piperonyl butoxide, and mixed-function oxidase (MFO) inhibitors in the strains where resistance was detected, we found that resistance diminished in all strains. For lambdacyhalothrin, the factor of synergism (FS) oscillated between 2 $\times$  and 3 $\times$ . The MBI and Girardot strains had an FS = 25 $\times$  for cyfluthrin and 255 $\times$  for deltamethrin, respectively. This involved the MFO system as a resistance mechanism in these strains to pyrethroids. The re-

sults indicate that insecticide resistance in *Ae. aegypti* is a problem of focal nature that should be treated independently.

**Resistance mechanisms in Mexican *Anopheles albimanus* using single insecticide, rotation, and mosaic strategies**

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A large-scale, 3-year field trial to evaluate insecticide resistance management and to investigate resistance gene frequencies of *Anopheles albimanus* populations was conducted in southern México. Before the intervention, a broad-spectrum resistance to DDT, pyrethroids (PYRs), organophosphates, and carbamates was detected by World Health Organization bioassays. Biochemical assays showed that resistance was conferred by glutathione S-transferase (GST), elevated esterases, monooxygenases (mixed-function oxidases [MFOs]), and altered acetylcholinesterase (AChE)-based mechanisms. Differences in resistance gene frequencies between villages and collection methods suggested the presence of subpopulations. During the 3-year study, significant variations in the enzymatic levels between the different resistance management strategies were detected. Elevated esterases and MFOs decreased in mosquitoes from villages under the continuous PYR pressure at the end of the intervention, indicating the presence of another resistance mechanism. The PYRs used in the rotation and mosaic strategies apparently selected for elevated esterase- and MFO-based mechanisms. The GST activity and altered AChE gene frequencies decreased in villages under the PYRs and rotation strategies by the 3rd year of intervention. Differences in MFO and GST gene frequencies were associated with sex; with females having higher levels of MFOs and males higher levels of GSTs. Resistant males with elevated esterases outnumbered resistant females by 2 to 1.

**A 3-year field evaluation of resistance management strategies in Mexican *Anopheles albimanus***

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Since 1995, we have undertaken annual rotations of unrelated insecticides (organophosphorus [OP],

pyrethroid [PYR], and carbamate [CAR]), spatial mosaic spraying of 2 unrelated insecticides in the same area (OP + PYR), and traditional spraying of a single insecticide (DDT or a PYR) to compare the efficacies of these strategies for resistance management. High levels of DDT resistance and low levels of resistance to OP, CAR, and PYR insecticides were detected before the intervention. The DDT resistance did not change in any of the treatments after 3 years of intervention, whereas glutathione *S*-transferase levels remained constant only in the DDT-treated areas. Decreases in susceptibility levels to the selected insecticide were detected in the rotation but these reverted soon after the application of the subsequent insecticide. In the mosaic, resistance to both insecticides increased and PYR resistance tended to stabilize by the 3rd year of application. In the PYR-treated areas, PYR resistance increased constantly after the 1st year of intervention. Multiple resistance mechanisms were present and some resistance mechanisms declined after the 1st intervention year; however, they fluctuated during successive sprayings. An altered acetylcholinesterase was the major resistance mechanism for OP and CAR, whereas monooxygenases and esterases conferred PYR resistance.

## POSTERS

### Scorpions of Oaxaca, México

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In the state of Oaxaca, México, a high index of scorpion stings exists each year. Although they are not always fatal, different symptoms do appear. Our objectives were to identify and to define the distribution of scorpions in the state. Biological materials were collected during entomological inspections made by state health vector program personnel from July 1997 to July 2000. Scorpions were preserved in 70% ethyl alcohol and sent to the Oaxaca State Public Health Laboratory for identification. A total of 3,725 scorpions including 4 families, 4 genera, and 12 species was identified from 97 (16.6%) of Oaxaca's municipalities. We identified species in the family Buthidae: *Centruroides infamatus infamatus*, *C. limpidus limpidus*, *C. elegans*, *C. nigri-manus*, *C. nigrovariatus*, *C. fulvipes*, *C. gracilis*, and *C. nigricens*; the family Vaejovidae: *Vaejovis* sp.; the family Diplocentridae (*Diplocentrus* sp.); and the family Iuridae (*Hadrurus* sp.). The most dangerous species were *C. infamatus infamatus*, *C. limpidus limpidus*, and *C. elegans*. *Centruroides infamatus infamatus* was the species with the highest density versus the rarely collected *C. nigricens* and

*Hadrurus* sp., with 0.02% of the collections. The broad distribution was of *C. infamatus infamatus*, which was found to be the most widely distributed on a monthly basis, coming from 64 municipalities.

### Morphometric characterization of *Anopheles (Nyssorhynchus) marajoara* from Venezuela

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*Anopheles (Nyssorhynchus) marajoara* was originally described in 1942, but later *marajoara* was considered a synonym of *albitarsis*. Current molecular evidence suggests that *An. albitarsis* is a complex of at least 4 species: *An. albitarsis* sensu stricto, *An. deaneorum*, *An. marajoara*, and a 4th species (species B), which has not been yet morphologically described. The available morphological keys for species identification of *An. marajoara* are based on mixed specimens within the *albitarsis* complex. To clarify the taxonomic status of *An. marajoara* in Venezuela and to determine diagnostic characters that can be used to update taxonomic keys, a morphometric study was carried out on populations of *An. marajoara* in Venezuela. To evaluate the diagnostic power of the characters identified among the populations of *marajoara*, the other 3 sympatric species belonging to the Argyritarsis Section: *An. argyritarsis*, *An. darlingi*, and *An. braziliensis* were included in the analysis. Selected wing, leg, and head characters of adult females were measured and analyzed on 195 females from 12 populations along the range of its distribution in Venezuela. The male genitalia of 5 populations were dissected and all characters were measured. Adult females of *An. marajoara* can be identified by hindtarsomere 5 white; sternum I with a sub-medial stripe of white scales; caudolateral scale tufts well developed on abdominal segment III; pre-humeral dark spot on wing costa 0.22–1.0 length of humeral pale spot; and length of dark band on hindtarsomere II 0.37–0.60 length of tarsomere. Resulting measurements on male genitalia components were compared to those previously reported for *marajoara* and *albitarsis*. In general, the range of variation for the characters measured are within the ranges previously reported for *An. marajoara*, except for the proportion length of gonocoxite divided by length of seta b, which showed a wider range of variation for the Venezuelan populations. The proportional length of gonocoxite divided by the width of gonocoxite for *marajoara* from Venezuela was smaller than the reported value for *albitarsis* sensu Linthicum. The diagnostic characters for identifying adult populations of *An. marajoara* will be useful for updating taxonomic keys to identify Venezuelan anophelines.

### Effectivity of Pirenona® 3-30N against *Musca domestica* larvae (Diptera: Muscidae)

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The housefly, *Musca domestica*, is a cosmopolitan insect, and frequently is found near human dwellings. Several methods have been used for its control; however, housefly resistance to all major groups of insecticides has been increasing. The objective of this study was to evaluate the toxicity of Pirenona® 3-30N to *M. domestica* larvae. The tests were conducted with small pieces of filter paper that were impregnated with 6 concentrations of Pirenona. After the papers were introduced into a small glass containers, ten 3rd-stage larvae were introduced in each test system and mortality was registered after 1 and 24 h of exposure. The data were analyzed statistically with linear regression, correlation, and determination coefficients. The mortality after 1 h of exposure was represented by  $b = -0.7671$ ,  $r = 0.4351$ , and  $r^2 = 0.4039$ ; mortality 24 h after application was represented by  $b = 1.9035$ ,  $r = 0.9545$ ,  $r^2 = 0.9079$ . The regression and correlation coefficients were not significant ( $P < 0.05$ ).

### Operational program to control *Chironomus plumosus* (Diptera: Chironomidae) in a wastewater plant

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After several years of suffering the presence of large swarms of *Chironomus plumosus* in the wastewater plant Agua Industrial de Monterrey, studies were carried out to identify the best tools for an operational control program. During 2000, no swarm of *C. plumosus* occurred, and no complaints were received at the water treatment facility. The best way to control these midges was to place an oviposition trap mesh to collect and eliminate the egg masses. Midge control was possible at the beginning of the life cycle. The operational program also included release and establishment of backswimmers, the use of light traps, and impregnation of surfaces to kill the adults.

### Entomological risk in a potential zone of ecotourism

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It is important not to expose tourists to health problems. In this study, we identified the insects that could pose entomological risks to human health in San Antonio Peña Nevada, Zaragoza, Nuevo León, México, an ecotourism zone. Every month samples were taken from aquatic systems and preserved in ethyl alcohol until identified in the laboratory. Diptera was the most important order of insects collected and included 25 genera. The most important species were *Culiseta inornata*, *Cu. particeps*, *Orthopodomyia alba*, *Culex restuans*, *Cx. quinquefasciatus*, *Cx. coronator*, and *Tabanus stygius*. These could become public health problems because they take blood. The 2nd most common order was Coleoptera with 7 species, followed by Hemiptera with 3 species, and Odonata and Ephemeroptera with 2 genera each, respectively.

### Diversity of flies in solid material in a wastewater plant

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The 1st step in the treatment of wastewater is the separation of solid material, which has high organic content and can become an ideal breeding site for flies. We studied solid material in 4 types: coarse particulate organic matter (CPOM) at the beginning of the wastewater treatment, plastic, CPOM in a dryer section, and material introduced in a container. A 1-kg sample was taken and all larvae were separated and preserved in ethyl alcohol. *Musca domestica* was the dominant species (98% of the total) and other fly larvae were *Fannia* sp., *Sarcophaga* sp., and *Stratiomyis* sp. The highest densities were found in the material from the container with 1,182 larvae.

### Selectivity of oviposition sites of *Aedes aegypti* under laboratory conditions

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Oviposition site selection by *Aedes aegypti* females was evaluated in ovitraps baited with temephos (Abate®), *Bacillus thuringiensis* var. *israelensis* (*Bti*) 10% (Bactimos®), methoprene 4% (Altosid®), poly (oxy-1,2-ethanediyl),  $\alpha$ -isooctadecyl- $\omega$ -hydroxyl 100% (Agnique® MMF), 3% chloric acid (Cloralax®), and distilled water as the control. The tests were carried out in a rearing cage where 20 gravid females were introduced; they were bloodfed daily and every 3rd day the red oviposition strips were separated and the collected eggs were counted until the last female died. Analysis of variance was used to find the significant difference among treatments. The highest number of eggs obtained was 914 in the control. Eggs in the other treatments were 824, 433, 341, 316, and 8 with *Bti* (824), methoprene (433), temephos (341), chloric acid (316), and a monomolecular film (8). Analysis of variance showed a significant difference among treatments ( $P = 0.05$ ). The difference was caused by Agnique MMF.

### Susceptibility of *Aedes aegypti* larvae to the historic, most commonly used, and alternative insecticides in Veracruz, México

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The insecticide resistance problem along with the absence of a domestic hygiene program in Veracruz State, México, have contributed to the high prevalence of dengue cases. Peridomestic water containers, such as 55-gal metal drums, cement tanks, and discarded tires, are the most important breeding sites for *Aedes aegypti*. The discarded tires are responsible for the build-up of this mosquito's population. To date, no report has been made of insecticide susceptibility in *Ae. aegypti* larval populations in Veracruz. The objective of this study was to determine the level of susceptibility of *Ae. aegypti* to the historic, most commonly used, and alternative insecticides against this mosquito species. Egg samples from 10 ecological sites of Veracruz State were taken to determine the mortality-concentration lines for biphenethrin, cypermethrin,

malathion, temephos, and DDT. We used the World Health Organization (WHO) bioassay methodology. The results were analyzed by probit analyses using WHO and the maximum likelihood methods.

### Comparison of developmental cycles of *Aedes aegypti* larvae exposed to sublethal concentrations of 2 formulations of *Bacillus thuringiensis* var. *israelensis*

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Understanding the developmental cycle of mosquitoes allows us to better manage control of these insects. In this research, the duration of the developmental periods of larvae exposed to sublethal concentrations of *Bacillus thuringiensis* var. *israelensis* using commercial formulations of Vectobac G & AS was determined. Concentrations of 30% lethal concentration ( $LC_{30}$ ),  $LC_{50}$ , and  $LC_{70}$  were used under laboratory conditions of 12:12 h light: dark, 28°C mean ambient temperature, and 26°C mean water temperature. Analysis of variance and Tukey multiple range tests were used to analyze the data. Developmental periods of 16.5, 15.5, 11.5, and 8.5 days were obtained for Vectobac G exposure for  $LC_{30}$ ,  $LC_{50}$ ,  $LC_{70}$ , and control, respectively. A significant difference between control and  $LC_{30}$  and  $LC_{50}$  treatments was found. Exposure to  $LC_{30}$ ,  $LC_{50}$ , and  $LC_{70}$  of Vectobac AS resulted in a developmental cycle of 7.5, 19.5, and 19.5 days, respectively, compared to 17.0 days for control. The  $LC_{30}$  results differed significantly from the  $LC_{50}$  and  $LC_{70}$  results and the control.

### Larvicidal effects of *Tagetes erecta* and *T. lucida* (Compositae) against the dengue vector *Aedes aegypti*

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Research on plant-derived molecules with killing effects on disease-carrying insects has intensified in recent decades. Presence of resistance or the avoidance of environmental pollution are 2 bases for alternate control measures. Local plants used for industries such as food sources, cosmetics, and traditional medicine were investigated in this study. *Tagetes erecta* and *T. lucida* supplied organic extracts with suspected larval mortality activity. Bioassays using 3rd-stage *Aedes aegypti* larvae exposed to a methanol extract of *T. erecta* showed a

median lethal dose (LD<sub>50</sub>) of 75.5 ppm and a LD<sub>90</sub> of 185 ppm. On the other hand, a hexane extract of *T. lucida* demonstrated mortality ranges of 6.6–100% for 90–130 ppm, respectively.

### Diet effects on egg hatching, life cycle duration, and gender ratio of *Aedes aegypti* and *Aedes albopictus* in Nuevo León, México

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Body size and longevity are responsible for producing highly efficient dengue virus vectors. *Aedes aegypti* and *Ae. albopictus* populations prevail in northwestern México, where dengue fever occurs. Eggs from paper strips collected in ovitraps were used and, therefore, the 2 species were mixed. They were hatched and reared on an optimal or suboptimal dry liver powder diet. The effect of both diets on the following biological parameters was assessed: hatching rate, life cycle duration, species proportion, and gender ratio. Emerged adults were fed with human blood and dissected to measure oogenesis. From a group of 3,605 eggs collected between March and July 2000, about 676 adults emerged. *Aedes albopictus* comprised 62.8% of the adults and *Ae. aegypti* comprised 37.2% of the adults.

### Age structure of *Aedes aegypti* and *Aedes albopictus* in Nuevo León, México

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*Aedes albopictus* was found in Nuevo León State in 1998. However, its role as an effective flavivirus vector is unknown in northeastern México. On the other hand, *Aedes aegypti* is a common dengue vector in this endemic area. To better understand and to prevent risks of dengue fever transmission by *Ae. aegypti*, studies focused on vectorial capacity variables in the field. We studied the age structure by determination of the blood digestion stage and ovarian development. The study site was Allende City, where 50 dwellings were selected to conduct human landing collections. Concurrently, female mosquitoes were aspirated indoors and outdoors to assess postfeeding resting behavior. Studies were conducted in September and October 2000. Human landing collections produced 86.4% *Ae. aegypti* and 13.5% *Ae. albopictus*. Age structure determinations

using abdominal appearance and ovarian development of both species resulted in following categories: 84% unfed and nulliparous, 8.2% parous, and 0.9% gravid.

### Mapping *Ochlerotatus albifasciatus* abundance in central Argentina with AVHRR/NOAA imagery

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*Ochlerotatus albifasciatus* is a Neotropical floodwater mosquito developing over short periods in temporary ground pools. In the Mar Chiquita depression, the adult mosquito population increases during the rainy season, and females irritate livestock and thus affect dairy production. Previous studies showed that higher densities were recorded in areas that periodically flood, where more larval sites are available. Normalized Difference Vegetation Index (NDVI) images calculated from Advanced Very High Resolution Radiometer (AVHRR) products were used to map areas with high probability of adequate *Oc. albifasciatus* breeding sites. Training sites for high, medium, or low abundance were defined as 3.3 × 3.3-km polygons over mosquito sampling sites, and were used on a supervised classification of National Oceanographic and Atmospheric Administration (NOAA)/AVHRR imagery. The first 3 components of a standardized principal components analysis on a NDVI time series represent statistical abstractions of the inherent variability of the original image set, and were used as bands in the classification. A maximum-likelihood classification resulted in a 78.6% probability of predicting areas with potential for yielding high mosquito densities. Results suggest that NOAA images were useful to identify those areas potentially more important for mosquito development or activity and where increases in the adult density could affect daily production.

### Abundance of adult *Ochlerotatus albifasciatus* in relation to habitat features

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An extensive area of central Argentina is periodically flooded by rainfall and river overflow, providing breeding sites for mosquitoes. Adult population explosions of *Ochlerotatus albifasciatus* irritate livestock, affecting milk and beef production. Although the species is a known vector of western equine encephalomyelitis virus, its popu-

lation dynamics on a regional scale is poorly known. This work studied factors affecting the spatial pattern of mosquito abundance. Adult mosquitoes were collected with light traps baited with CO<sub>2</sub> in 28 sampling sites in a 14 × 14-km study area south of Mar Chiquita Lake. Each site was defined according to its vegetation cover, terrain slope, cattle density, and distance to potential breeding sites. The spatial and temporal patterns of abundance suggested that this species preferred prairies and natural grasslands subject to periodic flooding vs. woodlands and agroecosystems. A discriminant function based on the proximity to *Salicornia* sp. and *Sesuvium* sp. prairies, distance to woodland, and land slope correctly classified 95% of the data having an average high (>500) or low (<500) mosquito abundance, and was validated using 6 sites located away from the study area. Using the discriminant function, a map of abundance distribution was created with the aid of a geographic information system.

#### Levels of insecticide resistance to organophosphates in field strains of *Aedes aegypti* from Venezuela

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As part of an insecticidal resistance surveillance program, resistance to several organophosphates (temephos, malathion, fenitrothion, and methyl pirimiphos) was investigated in 8 field strains of *Aedes aegypti* collected in 1997 and 1999 from Venezuela. The larvae were bioassayed using the World Health Organization standard procedure. All strains showed high susceptibility to temephos (<4-fold), fenitrothion (<3-fold), and methyl pyrimiphos (<9-fold), but were resistant to malathion (>36-fold). Malathion and temephos have been used extensively in Venezuela for *Ae. aegypti* control. Tests of adults revealed resistance to malathion in all strains. Biochemical studies of the synergists diethyl triphosphate and piperonyl butoxide suggested that malathion resistance was associated with the esterases and mixed-function oxidase mechanisms. Continuous monitoring of the insecticide resistance in these populations is fundamental for rational mosquito control and insecticide application programs.

#### Field evaluation of effectivity of *Bacillus sphaericus* against *Anopheles aquasalis* larvae in Venezuela

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*Anopheles aquasalis* is the main vector of malaria in northeastern Venezuela. A study of its biological control was carried out in Sucre State. The objective of this study was to evaluate the impact of the introduction of *Bacillus sphaericus* on larval populations of *An. aquasalis*. Aquatic breeding sites were a priori classified into 2 habitat categories: seasonal brackish mangrove with vegetation (*Avicennia germinans*), and semipermanent freshwater wetlands with herbaceous emergent vegetation (*Eleocharis mutata*). In field trials, 3 commercial concentrations (2, 3, and 4 g/m<sup>2</sup>) of a granular formulation (Vectolex® CG 7.5%) of *B. sphaericus* were evaluated in the 2 typical breeding sites of *An. aquasalis*. The 3 doses had the same effect against *An. aquasalis* larvae in breeding sites exposed to sunlight, low salinity, and with herbaceous emergent vegetation, producing a larval reduction greater than 90% during 14 days after treatment. In brackish mangrove breeding sites with *A. germinans*, the same concentrations were very effective in producing a larval reduction equal to or greater than 90% during 28 days after treatment.

#### Introduction to LECO hand-held dual mode ULV and thermal fogging machine for application of an adulticide with residual effect in dengue control

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An introduction to the new LECO hand-held dual-purpose ultra-low-volume (ULV) and thermal fogging machine and its use with an aqueous-based residual adulticide was presented. The purpose of this system is to provide immediate knock-down and kill of adult mosquito populations. The machine is also designed to extend residual protection within dwellings for a prolonged period of time to maintain *Aedes aegypti* mosquito density at reduced levels. The presentation outlined the concepts behind the system and gave results of recent field trials conducted in Nicaragua.

**Habitat characterization, dispersion and association of *Aedes albopictus* Skuse (Diptera: Culicidae) with other Culicids in Cuba**

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*Aedes albopictus* was first found in Cuba in 1995 in the municipality of Lisa within the province of Havana. Since then, this species has dispersed to other cities within the province. This paper discusses the distribution and dispersion of this species in the municipalities of Lisa and Boyeros. Most of the containers infested with *Ae. albopictus* were found outdoors and larvitrap (1/4 of automobile tire), which are widely used in Cuba's national *Aedes aegypti* surveillance program, were the "containers" most commonly infested. Artificial containers, including discarded tires and tin cans, were the next most commonly infested group of containers. These results suggest a possible competition for oviposition in larvitrap between *Ae. albopictus* and *Ae. aegypti*. Although *Ae. albopictus* was found breeding in association with *Aedes mediiovittatus* and *Culex quinquefasciatus*, the dominant species in Lisa and Boyeros, an increase in the number of containers infested by this species alone was ob-

served. These results may reflect interspecific competition between the recently introduced *Ae. albopictus* and long-established species, including *Ae. aegypti*.

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