

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

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**ABSTRACT.** The eighth annual Latin American symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 64th Annual Meeting in Sparks, NV, in March 1998. The principal objective, as for the previous 7 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 29 presentations that were given in Spanish by participants from 10 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies, studies of insecticide resistance, and molecular and ecological studies of vectors of dengue (*Aedes aegypti*), malaria (*Anopheles albimanus* and *Anopheles aquasalis*), and leishmaniasis (*Lutzomyia*).

**KEY WORDS** Mosquitoes, mosquito control, *Aedes*, *Anopheles*, *Culex*, *Lutzomyia*, resistance

### INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes and other vectors and promotes cooperation and interaction among professionals and students in this field. Although the majority of AMCA members are from the USA, an increasing number of members and almost one half of the contributors to the *Journal of the AMCA* are from outside the USA. To meet the needs of one segment of the international membership, the first Spanish language symposium was held at the AMCA Annual Meeting in 1991 to provide a scientific forum for scientists whose primary language is Spanish. In addition to addressing the language issue, the session promotes 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 state and federal government officials.

This publication includes summaries of 29 presentations that were given in Spanish by participants from 10 countries in Latin America. Topics addressed in the symposium included results from chemical and biological control programs and studies, studies of insecticide resistance, and molecular and ecological studies of vectors of dengue (*Aedes aegypti* (L.)), malaria (*Anopheles albimanus* Wiedemann and *Anopheles aquasalis* Curry), and leishmaniasis (*Lutzomyia*). Summaries of 6 previous symposia have been published (Clark and Suarez 1991, 1992, 1993; Clark 1995, 1996; Clark and Rangel 1997).

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Van Waters and Rogers (Ed Meehan), Zanus Corporation (David Sullivan), Zeneca (Eduardo Moreira), the Pan American Health Organization, and the Rockefeller Foundation. Funds from these sponsors partially defrayed travel and meeting expenses of symposium participants. Enthusiasm and interest for this symposium was high and this symposium will be a part of future meetings.

### SUMMARIES

#### Colonization and release of aquatic predators with Bactimos briquets for combined control of mosquito larvae

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In public health, more biorational approaches are needed to manage mosquito populations. One approach combines a specific chemical insecticide for mosquitoes and one or more biological control agents effective against these insects. The efficacy of 2 aquatic predators (the dytiscid *Laccophilus fasciatus*, and the damselfly *Archilestes californica*) and *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) (Bactimos®) briquets were evaluated as 2 mosquito larvae control strategies in plastic containers. A larval design with 4 strategies was developed: Bactimos added at a rate of one briquet per 9.29 m<sup>2</sup>, Bactimos plus 5 dytiscid adults, 5 dytiscids, and an untreated control. Natural oviposition of *A. californica* occurred in the containers, and they were removed from the control. Larval population densities were measured weekly by taking 10 dipper samples from each treatment. An analysis of variance was used to detect treatment differences. The treatments with lowest larval densities were the *B.t.i.* alone when the pooled data of mosquito larvae were analyzed and combined action of the bacteria-aquatic predators when larval *Culex* spp. densities were analyzed. No difference was found for

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the numbers of *Anopheles pseudopunctipennis* between the 2 strategies and the untreated control. We concluded that the natural mortality caused by aquatic predators used with biorational products offered good results in short- and long-term vector control programs.

### **Influence of filamentous algae on *Anopheles pseudopunctipennis***

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*Anopheles pseudopunctipennis* Theobald is a primary malaria vector in Mexico and breeds almost exclusively in association with filamentous green algal mats that occur in pools of receding rivers. To date, few experimental studies have investigated the ecological relationships between anopheline larvae and associated vegetation. The objectives of this study were to assess the effect of filamentous algal coverage on *An. pseudopunctipennis* survival and predation rates and its influence on pupation time and adult size; to assess the influence of the algae in the selection of habitat for oviposition by gravid females, and to assess the role of algae as a food resource. The study was carried out from June through September 1997 in a fish pond of Cacaohata, Chiapas, Mexico. For the first objective, a series of factorial experiments were undertaken with floating enclosures, testing 4 algal coverages and 4 fish predator densities (*Gambusia affinis* Baird and Girard). For the 2nd objective, a factorial experiment with floating oviposition substrates with and without algae of 2 genera (*Spirogyra* and *Cladophora*) was carried out. To accomplish the last objective, the feeding habits of larval *An. pseudopunctipennis* were assessed by analyzing the larval guts with 4'6-diamidino-2-phenylindole-2HCl (DAPI) staining.

Results demonstrated that *Spirogyra* aided the survival of *An. pseudopunctipennis* larvae by providing shelter from *G. affinis* predations, with a positive correlation between number of mosquito larvae and algal coverage and negative correlation with respect to fish density. Where high densities of fish predators were provided, the pupation time was increased and adults tended to be smaller in size. Significant differences occurred in mosquito survival in treatments with and without algae ( $F = 24.01$ ,  $df = 3$ ,  $P < 0.001$ ) and between presence or absence of fish ( $F = 168.08$ ,  $df = 3$ ,  $P < 0.001$ ). Similarly, the presence of algae favored oviposition by females ( $F = 6.76$ ,  $df = 1$ ,  $P = 0.01$ ), whereas no oviposition was observed in open water. No differences were observed in the selection of any algal species (*Spirogyra* or *Cladophora*) as oviposition

sites ( $F = 0.15$ ,  $df = 1$ ,  $P = 0.70$ ). Larval gut content analysis demonstrated that filamentous algae (*Spirogyra*) may be very important as a food source because they comprised 47% of all particles in the gut of 1,600 *An. pseudopunctipennis* larvae sampled. In conclusion, filamentous green algae provide oviposition substrates for *An. pseudopunctipennis* gravid females, as well possible as food and shelter for the mosquito larvae.

### **Community biological control of malaria using *Bacillus thuringiensis* var. *israelensis*-coconut in Piura, Peru**

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The number of malaria cases recorded by the Ministry of Health in Peru has been increasing since 1990. In 1996, 208,543 cases of malaria were confirmed, of which 49,962 were *Plasmodium falciparum*. Most cases of both types were reported from the states of Piura and Loreto. The purpose of this project has been to develop, test, and promote a set of techniques using *Bacillus thuringiensis* var. *israelensis* H-14 (*B.t.i.*) cultured in whole coconuts for larval vector control in malaria endemic areas. The goal is to eventually have these delivered in a sustainable fashion through direct community participation.

The intervention was implemented in the hamlet of Salitral, District of Sullana in Department of Piura state on the northern coast of Peru. Information about the community's knowledge and perception of malaria was developed to aid in the design of an effective educational strategy. Malaria was not viewed by the community as a serious problem. With this information, educational modules and appropriate tools (comics, videos, theater, and so on) to convey the messages were designed. We found that there was limited community participation and the school was the only dynamic entity in the community.

The schools appear to be a good location for the project to continue. A youth group, ABIMSA, which had been active in health education, formed the project's field educational component. After the educational intervention, a survey of 50 school children gave the following results: 100% of the brigade members knew the effects of *B.t.i.* action, 82% communicated their acquired knowledge to their families (using direct observation techniques), 60% knew all stages of the *B.t.i.* cultivation procedure, 54% correctly identified the malaria transmission cycle, and 25% were able to distinguish between *Anopheles* and *Culex* larvae. Correct knowledge about malaria rose from 10 to 32%. A

positive correlation was found between accurate knowledge of malaria transmission and the presence of a participating student in households. More than 80% of the residents surveyed were in favor of continuing the experience with the students in order to "better control the malaria problem" and to "share knowledge about the problem with the rest of the community."

In the coconut-*B.t.i.* produced by the community, the average number of *B.t.i.* spores per coconut was about  $2.2 \times 10^5$  spores/ml. The average larvicidal effect in ponds was 100% mortality of larvae at 6 and 12 h with a concentration of  $2.2 \times 10^3$  spores/ml. One group of the ponds received 2 coconuts and others received 4 coconuts and the *Anopheles albimanus* Wiedemann population decreased after 96 h. The percentage of mortality was between 80.7 and 89.4%. The anopheline larvae remained largely absent for 12 days after this period, whereas the larval population of the untreated ponds recovered normally.

The use of biological control of *Anopheles* vectors, supported by active community participation, could complement the National Malaria Control Program of the Ministry of Health, which focuses on case detection and limited use of chemical insecticides.

#### Residual activity of organophosphate insecticides on five types of surfaces on *Anopheles aquasalis*

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The surface where insecticides are applied can affect their performance and persistence so it is important to evaluate insecticidal activity on different types of surfaces. We evaluated different surfaces that are normally found in the field using the insecticides malathion, fenitrothion, fenthion, and methyl pirimiphos, and tested the surfaces and insecticides with the mosquito species subject to control. Residual activity of the organophosphorus insecticides was measured under laboratory conditions on 5 types of surfaces for 180 days. The surfaces (mud, mud with lime, cement, cement with lime, and painted cement) were sprayed with the insecticides as wettable powder at a concentration of 2 g/m<sup>2</sup>. We tested adult *Anopheles aquasalis* Curry from Yaguaraparo in Sucre State, Venezuela, following the method of the World Health Organization. We used the effectivity index (EI) where the EI = number of dead insects/number of exposed insects × time of exposure. Comparisons between types of surfaces and insecticides were made with the analysis of variance of Kruskal-Wallis. We

found significant differences between insecticides ( $H = 107.85$ ;  $P = 0.00001$ ;  $df = 9$ ) and between the types of surfaces ( $H = 9.658$ ;  $P = 0.046$ ). The greatest EI values were obtained for painted cement, and among the insecticides the most effective was methyl pirimiphos (2.53), followed by fenitrothion (0.52), fenthion (0.32), and malathion (0.22). Recent studies have found that populations of *An. aquasalis* from Sucre State were resistant to DDT and pyrethroids but were susceptible to organophosphorus insecticides. These facts and the results of this evaluation show that methyl pirimiphos is the best choice. It should be used in a plan of insecticide rotation not only because of its persistence and biological activity but also because it could be used to counteract pyrethroid resistance.

#### Residual activity of indoor application of ten insecticides against *Anopheles darlingi* in Itupiranga, Para State, Brazil

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In order to determine the residual activity of insecticides for the control of *Anopheles darlingi* Root, the principal vector of malaria in Brazil, 9 insecticides in 10 formulations were compared with DDT in Itupiranga, State of Para, Brazil, from March 1995 until June 1996.

Standard World Health Organization susceptibility tests with impregnated papers were performed with female mosquitoes from the study area. Cone bioassays were conducted on unpainted wood and mud panels and house walls sprayed with the following formulations: organochlorines: DDT wettable powder (WP) at 2 g active ingredient (AI)/m<sup>2</sup>; organophosphates: chlorpyrifos emulsifiable concentrate (EC) at 2 g AI/m<sup>2</sup>, fenitrothion WP at 2 g AI/m<sup>2</sup>, malathion latex paint formulation at 2 g AI/m<sup>2</sup>; carbamates: bendiocarb WP at 0.2 g AI/m<sup>2</sup>; pyrethroids: alpha-cypermethrin soluble concentrate (FW) at 40 mg AI/m<sup>2</sup>, cyfluthrin WP at 50 mg AI/m<sup>2</sup>, deltamethrin soluble concentrate (SC) at 25 mg AI/m<sup>2</sup>, etofenprox WP at 0.4 g AI/m<sup>2</sup>, lambda-cyhalothrin microencapsulated at 30 mg AI/m<sup>2</sup>, and lambda-cyhalothrin WP at 30 mg AI/m<sup>2</sup>.

Mortality in the susceptibility tests was 100% at the concentrations and exposure times utilized, with the exception of DDT and alpha-cypermethrin. In the bioassays on sprayed surfaces, etofenprox had a longer residual action than the other insecticides on wood panels (100% mortality for >12 months) and the longest residual action on house walls (>70% mortality for >12 months). Four other formulations (cyfluthrin WP, deltamethrin SC, lambda-cyhalothrin WP, and malathion paint) showed mortality of 90-100% for >12 months on wood panels,

but none of them lasted even 9 months on house walls. Results on mud surfaces were poor. Only cyfluthrin and deltamethrin gave mortalities of  $\geq 80\%$  for 1 month, and after 6 months only etofenprox gave a mortality of  $>70\%$ .

**Evaluation of deltamethrin, lambda-cyhalothrin, and etofenprox against *Anopheles darlingi* using the Colombian curtain in Brazil**

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Our findings demonstrate that although deltamethrin, lambda-cyhalothrin, and etofenprox were all found to produce good residual activity and successfully reduced indoor feeding, etofenprox was the most effective in reducing overall feeding and survival of fed mosquitoes. Although, overall mosquito exit patterns from lambda-cyhalothrin- and deltamethrin-sprayed houses indicated a lack of repellency, high exit rates produced by etofenprox together with highly significant mortality rates, suggests an excito/irritance effect. Although vector contact with etofenprox was reduced, fed and unfed mosquitoes still maintained a higher degree of contact to produce a significant mortality, thereby reducing feeding success. Finally, these results show that by an objective evaluation of vector-control relationships, a better understanding of the impact that insecticides have on vector behavior and their potential role in malaria transmission and control can be achieved.

**A two-year field evaluation of the impact of different resistance management strategies on insecticide resistance in *Anopheles albimanus***

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Since 1995, in southern Mexico we have undertaken annual rotations of unrelated insecticides (ROT), mosaic spraying (MOS) of 2 unrelated insecticides in the same area, and traditional spraying with a single insecticide to compare the efficacies of these strategies. The ROT scheme included an organophosphorus (OP) during the first year, pyrethroid (PYR) during the 2nd, and a carbamate (CA) that is currently being applied during the 3rd year. The MOS included the application of an OP

and a PYR in the same locations during the 3 years. Two controls where traditional spraying with DDT and a PYR were included. Susceptibility tests (World Health Organization [WHO]) and biochemical assays have been used to monitor the susceptibility of *Anopheles albimanus* populations and the resistance mechanisms involved. High levels of DDT resistance and low levels to OP, CA, and PYR insecticides were detected by the WHO discriminating dosage procedure before the intervention began. The resistance to DDT was caused by elevated glutathione-S-transferase (GST) levels. The DDT resistance decreased slightly after the first spraying of OP in the ROT, and OP and PYR in the MOS. However, DDT resistance increased again after the 2nd year, when the shift to PYR in the ROT and a 2nd year of constant use of OP and PYR in the MOS took place. The constant use of DDT and PYR in the controls increased PYR resistance quickly as compared to the ROT and MOS strategies. The higher increase in OP resistance in DDT-sprayed villages as compared to the ROT- and MOS-treated villages could be caused either because GST is responsible for a secondary OP metabolism, or because of intensive OP use for agricultural purposes in these areas. Both of these possibilities are now being investigated. Changes in CA susceptibility reflected slight inhibition of acetylcholinesterase by biochemical assays.

**Conventional and selective intradomestic application of DDT and Bendiocarb for the control of *Anopheles pseudopunctipennis* Theobald in southern Mexico**

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The main objective of the study was to evaluate the pre- and post-indoor feeding behavior and mortality of *Anopheles pseudopunctipennis* following contact with conventional and selective applications of insecticide on indoor sprayable surfaces of experimental houses. The fully treated house (FTH) as well as the selectively treated house (STH) contained standard dosages of bendiocarb (0.4 g active ingredient [AI]/m<sup>2</sup>). The selective application consisted of 2 0.8-m wide horizontal swaths of insecticide, the first starting at 0.85 m from the base of the walls and extending up to 1.65 m in width and the 2nd starting at the base of the inner roofs extending upwards. Residual activity on wood remained  $>90\%$  in both the FTH and STH for 3 months. Mortality due to contact with tin remained  $>75\%$  for 2 months in the FTH, whereas in the STH mortality could be observed only 1 month after insecticide spray. Vector-human contact in the

FTH significantly increased nearly 6-fold outdoors (49.2 bites/man/night [bmn]), while remaining nearly the same indoors (8.3 bmn) as compared with the pretreatment and untreated house (UH), respectively. In the STH, a significant increase of nearly 4-fold occurred in the indoor bmn (30.7) and more than a 3-fold increase occurred in the outdoor biting rate (56.2 bmn) in relation to the UH. No significant differences in mortality rates were recorded in outdoor and indoor biting mosquitoes, which ranged from 25.5 to 38.1% in both FTH and STH. Mortality in insecticide-treated houses was significantly higher than pretreatment and control levels (12.5–15.6%). Three hours after release in the curtained houses, 81.3 and 84.6% of *An. pseudopunctipennis* had exited the FTH and STH, respectively, compared to 77.7% in the UH. Despite low proportions of fed mosquitoes exiting houses between 2100 and 2400 h, significant differences were found in mosquito feeding success from FTH and STH (2.3 and 1.0%, respectively), as compared with UH (8.3%). Prefed and postfed mosquitoes continued to land within the preferred resting height range on sprayed surfaces. After mosquitoes had a blood meal, reductions in total resting time on sprayed surfaces were observed with respect to UH in the FTH and STH, from 1.4- to 2.9-fold, respectively. Finally, the present data demonstrated that a combination of the feeding status of the mosquito, the type of insecticide application, and the effect produced by the insecticide are complementary factors that are instrumental in determining mosquito contact with insecticide. This was different for bendiocarb and DDT.

#### Cross-resistance to Malathion in Cuban *Culex quinquefasciatus* induced by larval selection with deltamethrin

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A strain of the southern house mosquito, *Culex quinquefasciatus* Say, from Cuba was subjected to deltamethrin selection to evaluate the usefulness of this pyrethroid insecticide for mosquito control. A high level of resistance developed after 4 generations of selection. Little or no cross-resistance was observed to other pyrethroids (lambda-cyhalothrin and cypermethrin), a carbamate (propoxur), and 2 organophosphate insecticides (chlorpyrifos and methyl pyrimiphos), but high cross-resistance was found to the organophosphate Malathion. The high resistance to deltamethrin observed after only 4 generations of selection indicated that this insecticide was not a good candidate for long-term mosquito control in Cuba. This conclusion was based on the widespread Malathion resistance existing in Cuba and the cross-resistance between deltamethrin

and Malathion observed in the current study. Possible resistance mechanisms responsible for this phenomenon are discussed.

#### Cyfluthrin for the chemical control of *Aedes aegypti* in Buga, Colombia

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The incidence of dengue hemorrhagic fever is on the rise in Colombia. Vector control is the most important means of reducing dengue risk. Adulticide with chemicals using ultra-low volume (ULV) application has been the most widely used method for dengue control. The following were results of the ULV application of Cyfluthrin (SOLFAC® EC 50) with Maxipros® and with Stihl® backpack equipment. We carried out a presurvey that was then compared to the postsurvey after Cyfluthrin application. Results showed a reduction of 20.4% with the Maxipros treatment and 94.4% with the backpack. The average number of mosquitoes per dwelling in the Maxipros-treated area was reduced from 2.4 before treatment to 2.1 after treatment. In contrast, with the backpack treatment, the average number of mosquitoes per dwelling was greatly reduced from 2.8 to 0.1. The bioassays in both areas had the following mortality rates results: 24% with Maxipros and 100% with the backpack. These results indicate that the backpack method (internal treatment) is more effective in reducing adult *Aedes aegypti* density when used in the dwelling.

#### Low-volume insecticide spray as an alternative method for the control of *Aedes aegypti*

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The resurgence of dengue fever and the rise in the incidence of dengue hemorrhagic fever in Mexico are in part a consequence of the failure of current measures to control the mosquito vector, *Aedes aegypti* (L.). Indoor residual spray of insecticide might be a better tactic, but its feasibility is limited by the high costs and timing of application. The aim of this study was to optimize indoor residual spray of insecticide, applying it as a low-volume spray using a synthetic pyrethroid (lambda-cyhalothrin, OMS 3021, Icon® 10 CS) against *Ae. aegypti* in Huixtla, Chiapas, Mexico (15°09'18"N, 92°28'30"W; population 45,000). Low-volume spray consisted of the 50 to 100-µm droplets at 25 mg of active ingredient/m<sup>2</sup> of pyrethroid on indoor

walls at a rate of 215 ml/min. Within the city, insecticide was applied to houses on 25 blocks, whereas the houses on 66 surrounding blocks were left untreated.

The insecticidal effect was evaluated by its impact on larval aedine infestation indices as well as indices for adult mosquitoes and oviposition rates. The insecticide's residual effect on various wall surfaces was also determined. During the 55 wk of study, the first 34 wk allowed the assessment of the natural fluctuations of *Ae. aegypti* populations. Insecticide was applied during weeks 35–37, at a rate of 20 houses/sprayman/day. Each house was treated for an average of 10 min; including the actual spray, removal of furniture and house appliances, and protection of food. Insecticide residual effect on all wall surfaces tested caused mosquito mortalities  $\geq 75\%$  for up to 19 wk. All aedine indices were significantly reduced in insecticide treated vs. untreated areas, respectively: house index for larvae ( $\bar{x}$  arcsin = 0.075 and 0.297;  $t = 9.08$ ,  $P = 0.001$ ), Breteau index ( $\bar{x}$  arcsin = 0.083 and 0.367;  $t = 7.36$ ,  $P = 0.001$ ), house index for adults ( $\bar{x}$  arcsin = 0.04 and 0.47;  $t = 9.81$ ,  $P = 0.001$ ), mean adults per house ( $\bar{x}$  arcsin = 0.007 and 0.042;  $t = 4.77$ ,  $P = 0.0001$ ), and oviposition rates ( $\bar{x}$  arcsin = 0.192 and 0.864;  $t = 13.74$ ,  $P = 0.001$ ), except the container index ( $\bar{x}$  arcsin = 0.04 and 0.03 in treated vs. control areas, respectively,  $t = 1.54$ ,  $P = 0.013$ ). In conclusion, the pyrethroid applied by low-volume spray had a significant impact on the *Ae. aegypti* population. This vector control strategy could be implemented in particular during dengue outbreaks, because it can suppress adult mosquito populations for up to 12 wk.

### **Integrated control of *Culex quinquefasciatus* from sewers in Cali, Colombia using *Bacillus sphaericus***

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Storm sewers are the principal production sites for *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) mosquitoes in Cali, Colombia. Larviciding with chemicals has been widely used by the government throughout the country for reducing adult mosquito abundance. However, insecticide resistance in some mosquito populations and environmental concern suggest the need for greater balance in the program. We present preliminary results concerning the susceptibility of *Cx. quinquefasciatus* to Vectolex® and its impact on wild populations of *Cx. quinquefasciatus*. The baseline of susceptibility for *Cx. quinquefasciatus* to *Bacillus sphaericus* (strain 2362: Vectolex, Abbott Laboratories, Chicago, IL) was a median lethal concentration of 0.015 mg/L. A pilot field trial using 20 sewers each

treated with 5 g of granular formulations of Vectolex showed the following residual effect of mosquito mortality. Twenty-four hours after Vectolex application, the larval mortality was 96%. After 48 h and 8 days of treatment the mortality was 99%. We continued monitoring the residual effect of Vectolex that is particularly effective against *Culex* larvae.

### **Validation of the house and Maya indices to establish risk of *Aedes aegypti* populations**

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Better aedine indices are needed to correlate entomological risk of dengue transmission in the surveillance and prevention of dengue. Among the alternative indices to assess risk of *Aedes aegypti* (L.), the Maya index (MI) and premise condition index (PCI) have been recently evaluated. In these, the MI was less sensitive than the PCI to predict houses with high risk of presence of larvae or adults of *Ae. aegypti*. In the present study, we validated the PCI in southern Mexico (Ciudad Hidalgo, Chiapas; 14°41'N, 92°09'W, population 15,000) where dengue transmission is high, and tested a modification of the MI. The study was carried out from July to October 1996, collecting *Ae. aegypti* larvae and adults between 0900 and 1400 h in 717 randomly selected houses. The indices assigned levels of high, medium, and low risk of *Ae. aegypti* as follows. The PCI used the appearance of the house ("weariness" of roof and floor and openings on the wall), level of tidiness, and amount of shade of the backyard. The MI categorized each house by its "hygiene," that is, the number of disposable containers, and its "breeding risk," that is, the number of controllable containers, dividing both types of containers by the mean number of containers of that type in the community. The weighted MI (MI<sub>w</sub>), was the same as the MI, except that both container types were divided by the mean number of positive containers of that type in the community. For comparison, the Breteau index (BI) was calculated.

The PCI probabilities for houses at medium or high risk of *Ae. aegypti* larvae were 4.36 (95% CI = 1.33–14.29) and 15.19 (95% CI = 4.55–50.60) greater than houses with low risk. For adults the probabilities for houses at medium or high risk and for adults the probabilities for houses at medium or high risk were 1.69 (95% CI = 0.85–3.34) and 3.57 (95% CI = 1.75–7.34), respectively. Although the MI could not associate risk of presence of mosquito

larvae or adults, houses classified by the  $MI_w$  at medium and high risks were 2.35 (1.47–3.70) and 2.33 (1.52–3.59) times at greater risk to have containers with *Ae. aegypti* larvae and for adults, houses classified at high risk were 1.71 (1.17–2.50) greater risks than houses classified at low risk. Agreement for the PCI and  $MI_w$  was slight (39%,  $K = 0.06$ ,  $P < 0.05$ ). The BI was 0.50. In conclusion, the validity of the PCI to predict the presence of *Ae. aegypti* larvae or adults was demonstrated. In addition, the  $MI_w$  provided a better approximation for the risk of *Ae. aegypti* breeding in houses than the unweighted MI.

**A study of the entomological risk factors (intra- and peridomiciliary resting habits of *Aedes aegypti*) for the transmission of dengue**

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Although *Aedes albopictus* (Skuse) has been documented to have infested Guatemala and El Salvador, the principal vector of dengue in Central America and Mexico is *Aedes aegypti* (L.). The objectives of this study were 2-fold: to study the intra- and peridomiciliary resting behavior of *Ae. aegypti* in the absence of insecticide application, and to compare the age structure and preferred resting sites of this mosquito.

Our results demonstrated that the *Ae. aegypti* (male and female) intra- and peridomiciliary resting activity peaked between 1400 and 1600 h in bedrooms and wash basins/barrels (peridomiciliary), respectively. Similarly, proportions of gravid females (intra- and peridomiciliary) generally increased to more than 50% during the same period. Kitchens and hallways were found to provide the least favorable resting sites during the sampling period, 0800–1800 h. These results illustrate that by more effectively targeting vector behavior, an alternative to traditional control strategies can be achieved.

**Control of *Aedes aegypti*: an educational strategy involving the participation of medical students from the University of Panama, 1992–1997**

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For 5 consecutive years, the Microbiology Department in the Faculty of Medicine at the University of Panama introduced a dengue prevention protocol as an educational strategy to incorporate students' participation to help in solving a public health problem: the reinfestation of Panama with

*Aedes aegypti* (L.). Since 1985, the mosquito, which transmits dengue, has reinfested Panama. This report documents the activities carried out by 10 groups of participating medical students (600) to improve the consciousness of the university students in the prevention of dengue and to eliminate active *Ae. aegypti* breeding sites. Various types of water-filled containers were found to be positive for the larvae or pupae of *Ae. aegypti*, including plastic vases, bottles, cans, air conditioner pans, and irregular structures in buildings; all were easy to eliminate. Various methods were used to inform the community, mostly involving speeches, brochures, illustrative boards, and seminars. Satisfaction and increased awareness were evident at the end of each semester, when the students presented their recommendations to other groups.

**Eradication of *Aedes aegypti* from the Island of Taboga with community participation: the diagnostic phase**

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In 1995, the dengue surveillance program in Panama reported 14 cases of dengue fever caused by dengue-3 on Taboga Island. This situation was of particular interest to the National Committee Against Dengue. The epidemiologic implications created a basis for creating a program with community participation against *Aedes aegypti* (L.) on Taboga Island.

With the objective of elaborating the necessary strategies to completely eliminate *Ae. aegypti* on the island, we conducted a diagnostic survey of the sanitary situation. During this period, we completed entomological, community, and sanitary surveys. We interviewed teachers, civil authorities, and personnel of the National Institute of Aqueduct and Sewerage.

The 625 existing landed properties on the Taboga Island were evaluated and we found that the principal mosquito breeding sites were in water stored for domestic use and inside summer houses that were closed for more than 1 week. The entomologic indices (house, Breteau, pupal, and the different density of the larval stages) reflected that breeding in unserviceable sites was a secondary problem.

The results of the community survey showed that the principal problem that the residents had was an undependable water supply. The strategy to eliminate *Ae. aegypti* breeding involves use of water reserve tanks with covers. The community must agree to acquire, use, and maintain those tanks. The owners of summer houses who have water tanks indoors must agree to use tank covers indoors.

These strategies were based on the results of the field survey.

**The eradication of *Aedes aegypti*:  
returning to the past or looking to the future**

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The Americas are now facing the emergence of dengue and its severe form, dengue hemorrhagic fever (DHF), which is rapidly becoming a major public health problem in the hemisphere. *Aedes aegypti* (L.), the principal vector of dengue virus in the Americas, is widely distributed in urban, peri-urban, and even rural areas throughout the region. In the early 1960s, this species was partially eliminated from the hemisphere and its eradication is now proposed as the solution for the transmission of dengue and the elimination of the risk of DHF in the Americas. The emergence of dengue and DHF has prompted an unusual response among public health institutions and the eradication proposals are justified based on the partial success of eradication in the past and the assumption of its feasibility in the present. The conditions that made the elimination of *Ae. aegypti* possible in the past have changed, and the tools available for the control or eradication of the species have not improved or increased. Social issues that modified the natural niche of the vector have increased the vector's density and dispersal. Urbanization, air travel, human migration, production of nonrecyclable goods, commerce, and poor public services in growing metropolitan areas are among the most important determinants of the expansion of *Ae. aegypti*'s range and are factors impeding our ability to achieve eradication. The control strategy for this vector is based on elimination of production sites and larvicidal application in expanding urban areas. Clean-up campaigns do not always target the most productive or stable breeding sites, and their organization is expensive, labor intensive, and relies on municipal initiatives. Larvicides have partial coverage, short impact, and demand many human resources. Insecticide spraying is expensive and has a limited impact. Eradication is conceived as a vertical strategy focused exclusively on the vector and it will certainly compete for the scarce financial and human resources in related public health programs. The issues of priority, vulnerability, political compromise, and costs involved in such a task as well as the problems and obstacles are discussed in detail. If there are reasons to engage in a major task such as eradication, there are also temptations and experiences of the past that should caution us to consider the potential consequences of such a program.

**Larvicidal impact of two formulations of *Bacillus sphaericus* (strain 2362: Vectolex CG, Spherimos liquid) on *Anopheles albimanus* larvae in natural habitats in Nicaragua in 1996**

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A study to evaluate the larvicidal impact of 2 formulations of *Bacillus sphaericus* (strain 2362, Vectolex<sup>®</sup> [h5a5B, CG] and Spherimos liquid) was carried out in Nicaragua. Six *Anopheles albimanus* Wiedemann breeding sites in an extensive malarious zone of Managua near the International Airport and located parallel to Xolotlan Lake were selected for study. Two of these sites were treated with Spherimos; one received the minimum dose (0.3 ml/m<sup>2</sup>) and the other the maximum dose (0.6 ml/m<sup>2</sup>). Spherimos was applied using a Hudson X-pert sprayer with 8002 nozzle. Two were treated by hand with Vectolex; one received the minimum dose (2 g/m<sup>2</sup>) and the other one received the maximum dose (5 g/m<sup>2</sup>). The remaining 2 sites were used as untreated controls.

The results indicated that both Spherimos or Vectolex can reduce larval *An. albimanus* populations from 95.3 to 100% at 72 h after application. During the study no significant differences were observed on the efficacy of dosage and formulation used. The physical and chemical factors of breeding places appear to have no influence on larvicidal activity of these formulations.

**Field evaluation of the lambda-cyhalothrin 10% WP to replace DDT for malaria control in Mexico**

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The North American Regional Plan includes discontinuation of DDT use for malaria vector control. Mexican authorities have therefore started evaluation of alternatives to DDT, such as the pyrethroid insecticide lambda-cyhalothrin (ICON 10% wettable powder [WP]) which has already been adopted for house-spraying against malaria vectors in over 30 countries. In an area of persistent malaria transmission in coastal Michoacan State, we sprayed approximately 5,000 houses with lambda-cyhalothrin (30 mg/m<sup>2</sup> in 1997). When interviewed after spraying, 97% of housewives were pleased that the treatment was effective against cockroaches, scorpions, and other domestic pests, and all house-holders accepted the regular spraying of houses with ICON 10 WP. Susceptibility tests with lambda-cyhalothrin (0.1% diagnostic concentration) showed >95%



mortality of *Anopheles albimanus* Wiedemann and *Anopheles pseudopunctipennis* Theobald. Among nearly 14,000 residents of sprayed houses, the malaria incidence was reduced by 37% during 1997 compared to the previous year when DDT was sprayed.

**Detection of resistance mechanisms in *Aedes aegypti* from Cuba and Venezuela: standardization of the methods**

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Five strains of *Aedes aegypti* (L.) larvae, one from Santiago de Cuba, Cuba, and 4 from the Venezuelan states of Apure, Aragua, Miranda, and Tachira, were bioassayed for sensitivity to the organophosphorus insecticides (temephos, Malathion, methyl pyrimifos, and chlorpyrifos) and the pyrethroids (deltamethrin, lambda-cyhalothrin, and cypermethrin). DEF and peronyl butoxide (PB) synergists were used to assess the possible involvement of esterases or oxidases as resistance mechanisms. This was the first time that biochemical assays were used to test for resistance in *Ae. aegypti* from Cuba. We standardized the techniques to detect elevated esterases and glutathion-S-transferase. The Venezuelan strains showed low levels of resistance to the organophosphate insecticides temephos and Malathion. Conversely, the Cuban strain showed moderate and high levels of resistance to temephos and Malathion, respectively. All of the strains showed low resistance levels to the pyrethroid insecticides. Polyacrylamide gel electrophoresis tests were performed to determine what kind of esterase was involved in the resistance. A highly active stained band named A4 was found in all the resistant strains, but not in the susceptible strains.

**Influence of highly active esterase in pyrethroid resistance in *Culex quinquefasciatus* from Cuba**

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A strain (SP6) of *Culex quinquefasciatus* Say collected from Santiago de Cuba, Cuba, was selected during 6 and 4 generations with lambda-cyhalothrin and deltamethrin. Different levels of resistance to those insecticides were observed when compared to the susceptible reference strain (S-

Lab). Cross-resistance to Malathion was also detected. Lethal concentrations inducing 50% mortality decreased when bioassays were conducted in the presence of the synergist DEF, an inhibitor of esterase enzymes. This represents a decrease in the resistance factor of more than 90%, indicating that elevated esterases are playing an important role in resistance to this pyrethroid. All SP6 mosquitoes showed an identical esterase pattern after polyacrylamide gel electrophoresis (B1, A6, B6). Crossing and back crossing experiments between SP6 and S-Lab strains showed that B1 and B6 esterases are coded by different genes, and that A6 and B6 esterases are linked, located just 6.7 recombination units apart. Resistance to pyrethroid was inherited in a multifactorial and incomplete dominant mode, due mainly to the influence of B1 and A6B6, and probably the action of other resistance mechanisms. Inhibition studies using the purified esterases showed this potential capacity to react with pyrethroids, especially esterase A6.

**Population genetic structure of *Aedes aegypti*, the vector of dengue in Mexico, using the RAPD-PCR**

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In 1997, Mexico experienced nearly 50,000 cases of classical dengue and about 1,000 cases of dengue hemorrhagic fever. This reinforced the importance of understanding the role of virus serotypes and mosquito vector population genetics, as a molecular approach for explaining the changing epidemiology of this emerging vector-borne disease. Vector control efforts and other environmental factors have produced a reduction in the genetic variability in *Aedes aegypti* (L.). Analysis of population genetics will provide information about the breeding structure of *Ae. aegypti* in Mexico. This will help us understand the relationship between the vector and dengue transmission, which will be invaluable in vector competence studies. The purpose of this study was to define the local breeding structure of *Ae. aegypti* in geographically separated areas of Mexico using rapid analysis of polymorphic DNA-polymerase chain reaction (RAPD-PCR) methodology. Mosquito larvae were collected in dengue endemic areas in 6 states, mainly from the Pacific coast areas of Oaxaca, Chiapas, and Guerrero, and adjacent to the Gulf of Mexico in Veracruz, Nuevo Leon, and Tamaulipas. Larvae were collected from peridomestic containers (55-gallon drums, discarded tires, 5-gallon buckets, and others) in backyards at 3 or 4 cardinal points in each

city. Mosquitoes were reared to the adult stage and frozen at  $-70^{\circ}\text{C}$  for DNA analysis. We tested 35 primers for RAPD-PCR and selected 7 of them. We found 55 polymorphic loci in 15 individuals using the analysis in agarose gels. Our preliminary results showed an  $N_m = 0.019 \pm 0.019$  ( $F_{ST}$ ) estimated according to Lynch and Milligan, which was smaller than the  $N_m = 0.030 \pm 0.012$  ( $F_{ST}$ ) estimated according to Wright for the northern and southern areas of Monterrey in Nuevo Leon. These results suggest that a larger sample size is needed to estimate gene flow more accurately; therefore, we will analyze this sample using acrylamide gels, which will give a higher number of polymorphic markers.

#### Variability in mitochondrial DNA in populations of *Anopheles albimanus* in Guatemala

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*Anopheles albimanus* Wiedemann is considered the principal vector of malaria in Central America, parts of South America, and the Caribbean. The species shows great variability in characteristics associated with its vectorial capacity. The species is considered a poor vector of malaria because it is more zoophilic than anthropophilic, more exophagic than endophagic, and more exophilic than endophilic in its behavior with humans. Morphologic variants have been reported in all stages and their feeding behavior varies with season and geographical location. Differences in their biting hours are more related to the human activity that surrounds them than to their natural circadian rhythms. It is not known if their role as vectors is determined by the high densities of mosquitoes or the existence of a species complex, with some species having a higher potential to transmit malaria. Little is known about the molecular genetics, population dynamics, and phylogeny of *An. albimanus*. Finding genetic markers that help identify malaria vectors is important. Proper identification combined with epidemiologic studies and transmission studies may have an impact on malaria control efforts.

There is great interest in developed countries in developing transgenic mosquitoes. To undertake this type of manipulation, it is necessary to know the basic genetic structure and population genetics of these mosquitoes. Guatemala has developed the infrastructure and expertise to undertake these types of studies.

Polymorphisms in mitochondrial DNA (mtDNA) are being increasingly exploited for the study of genetic relationships among closely related organisms. The simple and uniform organization, lack of recombination, maternal inheritance, and relatively

rapid rate of change make mtDNA a valuable tool for the study of molecular evolution and phylogenetic relationships. Numbers of maternal clones are indicative of founding population size and thus provide evidence of the existence of severe population bottlenecks. Maternal lineages can be traced and the phylogenies of existing populations derived independently of founder effects or adaptation. This evidence cannot be inferred from studies of nuclear markers that are biparentally inherited and recombine. Our ultimate goal in the mitochondrial DNA project is to trace the historical establishment of *An. albimanus* throughout Latin America.

We studied the mitochondrial DNA in the ND5 gene of *An. albimanus* from 3 malarious regions of Guatemala. Mitochondrial DNA fragments were amplified with primers designed from other insects and examined for variability with a technique called SSCP (Single Stranded Conformation Polymorphisms). A total of 1,236 *An. albimanus* was analyzed and 34 different ND5 haplotypes were identified from the entire country. The southern region of Guatemala presented 26 different haplotypes, the northern region presented 24, and the eastern region had 18 different haplotypes. The Shannon diversity analysis depicts the northern region of the country with the highest diversity ( $H = 3.478$ ), followed by the southern ( $H = 2.9364$ ) and eastern regions ( $H = 2.5171$ ). Analysis also indicates that the diversity of haplotypes among individuals within collections accounted for most of the diversity in each region (89% for the eastern, 82% for the northern, and 72% for the southern region). The diversity among collections within a site contributed to the diversity of the region only by 8.6% in the northern region, 4.5% in the southern region, and 0.3% in the eastern region. These results suggest that collections within a region were very uniform in diversity. Diversity between sites accounted for 19% of the total diversity in the southern region, 11% in the eastern region, and 90% in the northern region. This suggests that more genetic variation occurred between sites in the southern region compared to the other regions of Guatemala.

#### Distribution of *Anopheles aquasalis* in the Caribbean region: vicariance or dispersal

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*Anopheles aquasalis* Curry occurs from Brazil to Nicaragua, Ecuador, Trinidad and Tobago, and the Lesser Antilles. Studies of genetic structure carried out in Venezuela, Trinidad, and Barbados showed that mean heterozygosity per locus ( $H$ ), number of alleles per locus, and Wright's F statistics did not exhibit significant variation. However, the proportion of polymorphic loci ( $P$ ) was significantly lower

for populations from Barbados (11.15%) than for those from Venezuela (34.42%) and Trinidad (32.5%). This finding and an absence of rare alleles could be an indication that a major reduction in the gene pool occurred in the evolutionary history of the population from Barbados. This report presents a hypothesis of historical reconstruction to explain how the present patterns were formed. The Middle America–Caribbean region is extremely complex but models of Caribbean evolution have attempted to reconcile existing geological data with known or inferred tectonic plate motions. This model is a preliminary framework and it assumes a Pacific provenance for the Caribbean Plate, that the Lesser Antilles were more closely tied to South America during their development from the Late Eocene to the present time, and Pleistocene climates and changes in sea level exerted a powerful influence on zoogeographic patterns of distribution. The current population in Trinidad is suggested to be a fragmented biota, or vicariated from Venezuela; whereas the population in Barbados required dispersal events across intermediate islands by a stepping stone model.

#### Mosquito–phytotelmata biogeography in Venezuela using parsimony analysis of endemism

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We studied the association between immature mosquitoes and aquatic habitats in plants or part of plants (phytotelmata) from 17 localities in national parks and natural monuments in Venezuela. A total of 60 mosquito species was collected in a 3-year period from 47 host-plant species. With 2 exceptions, all other mosquito species collected were restricted to phytotelmata. Different degrees of mosquito–plant associations were observed, with the highest specificity existing at the genus and subgenus levels. We ran a parsimony analysis of endemism (Wagners method; Henning86 and NONA) using a 17 (localities as taxa)  $\times$  60 (mosquito species as characters) binary matrix. The final strict consensus cladogram (from 28 trees) showed 2 main, well-supported groups named Guayanes (Guayana Shield) and Andino (Los Andes), and another group not as well supported called Cordillerano (north-central Cordillera). Alternatively, using a semistrict consensus cladogram we found 7 groups, which can be split into 2 main groups: Guayanes and Cordillerano. The first group had 3 monophyletic subgroups (Amazonico, Gran-sabanico, and Guayanes), whereas the 2nd group had 4 subgroups (Andino, Perijano, Caribeo, and Centro-oriental). These results support the hypothesis that

the Guayana Shield was the ancestral center of speciation, followed by the Andean Region. The fauna from the Central Cordillera is derived from the latter and is evolutionarily more recent. This study represents a first attempt to understand the current distribution and evolutionary history of this insect group in Venezuela and adjacent areas.

#### Characterizing anopheline embryogenesis: clarification and permeabilization of initial assays

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The construction of transgenic mosquitoes that are refractory to a *Plasmodium* infection is an alternative to the control of malaria that has been the subject of a great deal of effort. In this sense the knowledge of the vector's embryonic development is extremely important, although it has been surprisingly neglected. Transgenic mosquitoes are expected to come from the injection of exogenous DNA into embryos and the consequent integration of this material into polar cells. Hence, detailed knowledge of early embryogenesis, including time and position of polar cell formation, is essential. Knowledge of wild mosquito embryogenesis as a whole will also be important to the characterization of mutants that are currently being isolated and of transgenic lines to be generated.

The recent availability of Neotropical anopheline colonies has made the study of embryogenesis possible; however, the melanized and sclerotized nature of the eggs of these mosquitoes still poses a problem. Thus, different methods have been used with the goal of clarifying and permeabilizing anopheline eggs.

The clarification method chosen is enabling the definition of the major morphogenetic movements of this long germ band embryo, as well as the establishment of a parallel with *Drosophila melanogaster*. These analyses have been made with confocal microscopy, because clarified anopheline eggs proved to be autofluorescent. Among different methods that are being tested for the permeabilization of anopheline eggs, the one classically used for the dechoriation and devitellinization of *Drosophila* eggs was not effective when applied to anophelines, as verified by scanning electron microscopy.

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**Population dynamics of *Lutzomyia evansi* and *Lutzomyia longipalpis* (Diptera: Psychodidae) in an ancient endemic focus of visceral leishmaniasis in Venezuela**

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Knowledge of the spatial distribution and behavior of sandfly vectors contributes to the understanding of the epidemiology of leishmaniasis. Information on the abundance and temporal distribution of vectors permits public health officials to predict transmission seasonality and assists in planning control strategies. Additionally, through the integration of entomological data and epidemiological data on humans and reservoirs from different defined ecotypes, an approach to the stratification of the leishmaniasis may be attempted. *Lutzomyia evansi* (Nunez-Tovar) and *Lutzomyia longipalpis* (Lutz and Neiva) recently have been found to be infected with *Leishmania (Leishmania)* spp. in Guayabita, Venezuela (20°10'N, 28°67'W), an ancient endemic focus of visceral leishmaniasis ecologically situated in a premontane dry forest. In this locality, 12.4% of humans were leishmanin positive and 15.5% of the dogs were seropositive. Monthly collections of phlebotomine sandflies were carried out using a fluorescent light-baited Shannon trap (January 1993–June 1994) and Centers for Disease Control (CDC) miniature light traps (May 1993–June 1994). A total 3,230 males and 6,091 females from 11 phlebotomine sandfly species was collected. *Lutzomyia evansi* was the dominant species (86.4%), 10-fold more abundant than *L. longipalpis* (10.67%). The 2 vectors markedly alternated seasonally: *L. evansi* peaked during the rainy season (May–November) and *L. longipalpis* peaked during the dry season (December–April). Plots of the abundance of both species and abiotic environmental factors (temperature, relative humidity, and rainfall) were correlated to determine the weight of each of these covariates. The results are compared with observations made elsewhere.

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**Response of Leishmaniasis vectors to various pesticides in Venezuela**

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Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) are endemic in Venezuela, where

*Lutzomyia ovallesi* (Ortiz) and *Lutzomyia longipalpis* (Lutz and Neiva), respectively, are recognized as vectors of these diseases in the country. Because both species enter houses, a control program based on the use of insecticides, indoor spraying, or impregnated curtains might be applicable. To determine the susceptibility to different insecticides, a laboratory (S) and field strain of *L. longipalpis* and a field strain of *L. ovallesi* were tested for their susceptibility to organochlorine (DDT 2%), carbamate (propoxur 0.01%), organophosphate (Malathion 2%, fenitrothion 1%, and methyl pirimiphos 1%), and pyrethroid (deltamethrin 0.06%, lambda-cyhalothrin 0.06%, and permethrin 0.2%) insecticides. Susceptibility to the insecticides tested was evaluated in the field population and compared with a reference strain of *L. longipalpis*. The 95% lethal time (LT95) to propoxur and Malathion insecticides for the field population was lower than the LT95 for the laboratory reference strain, showing high susceptibility to these compounds. A low level of resistance at the median lethal time (LT50) (<3-fold) was found for the fenitrothion, pirimiphos methyl, and permethrin insecticides, but no resistance was detected at LT95. No significant resistance at LT50 and LT95 was detected for the pyrethroid insecticides. A preliminary field trial using lambda-cyhalothrin (25 mg active ingredient [AI]/m<sup>2</sup>) sprayed in interior walls produced a reduction in the number of indoor sandflies for 2 months duration. Simultaneously, biological assays to test the residual effect of this insecticide on wood, clay, and block walls were made. Five, 69, and 131 days after the spraying, 3–6 batches of 20 laboratory-reared female sand flies were exposed for 30 min to sprayed walls. An average of 99, 39, and 5% mortality was obtained, respectively. The highest mortality was observed among sand flies exposed to sprayed wooden walls. Further trials are planned to design an improved strategy of control.

**Intra- and interspecific competition between *Aedes aegypti* L. and *Culex pipiens* L. (Diptera: Culicidae) in the laboratory**

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One component of population dynamics is competition. It is a very important factor in regulation of natural populations but often is forgotten in the control of mosquito larvae. Competition has been evaluated by studying larval mortality, growth, biomass, and fecundity. The objective of this study was to determine intra- and interspecific competition between *Aedes aegypti* (L.) and *Culex pipiens*

(L.) in the laboratory. The tests were carried out in glass containers with 750 ml of nonchlorinated water with 10, 20, 40, 80, and 160 1st-stage larvae of *Ae. aegypti* and *Cx. pipiens* with 5 replicates of each density. Every 3rd day, mosquito larvae were fed and when pupae appeared, they were placed in a cage where adults emerged. Data were collected on larval mortality, number of adults, sex ratio, and size of body, wing, and 3rd leg. Intraspecific competition was analyzed by linear regression and an isoclines model using the number of adults produced. Intraspecific competition was stronger in *Ae. aegypti* because larval mortality, number of pupae, wing size, 3rd leg size, and number of adults exceeded that of *Cx. pipiens*, but more *Ae. aegypti* females were produced. Finally, examination of the isoclines reflected that species coexisted at the point where the isoclines crossed.

### Some chemical factors of larval ecology of *Anopheles aquasalis* and *Anopheles pseudopunctipennis* in Venezuela

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Field surveys of mosquito breeding sites in coastal areas of Sucre State, Venezuela, were carried out in the dry and wet seasons of 1995. The sites were classified a priori into 5 habitat categories. At each site samples for the 2 malaria vectors, *Anopheles aquasalis* Curry and *Anopheles pseudopunctipennis* Theobald, were taken. Selected chemical factors of the habitat were measured or estimated; a water sample was collected for subsequent analysis and 25–30 dips were made for mosquito larvae. Principal components analysis revealed that the occurrence of *An. aquasalis* larvae in both the wet and dry seasons was positively associated with alkalinity or pH and negatively associated with dissolved oxygen. These results were confirmed by Spearman rank correlations. The occurrence of *An. pseudopunctipennis* larvae in the dry season is positively associated with pH and negatively associated with dissolved oxygen and salinity. During the wet season, flooding eliminated the typical *An. pseudopunctipennis* larval habitat; larvae were rarely encountered and only occurred in irrigation canals and streams with filamentous algae (*Spirogyra* spp. and *Cladophora* spp.).

### Melanization of oocysts and sporozoites of *Plasmodium vivax* in vector populations of *Anopheles aquasalis* in Venezuela

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*Anopheles aquasalis* Curry is an important vector of vivax malaria in eastern Venezuela. During

a study of susceptibility of this species to *Plasmodium vivax* carried out in vector and nonvector populations from eastern and central Venezuela, melanization of oocysts and sporozoites was observed in laboratory-reared (F<sub>1</sub>) progeny of field-collected females, experimentally infected from a vivax malaria patient. Sixty-one percent of the oocysts in mosquitoes showed melanotic encapsulation. The mean proportion of melanized oocysts was 0.40 per mosquito, showing a wide range (0.00–1.00). The melanotic parasite population was not normally distributed in mosquitoes. A correlation analysis showed that no association existed between the number of malaria gametocytes ingested and the proportion of melanotic oocysts. No association was found between the number of melanocytic oocysts and the number of developed oocysts, but a strong positive linear correlation was found with the age of the infection, that is to say, the number of melanized oocysts increased with time. Melanized parasites were observed at day 5. Melanotic sporozoites were observed in the hemocoel and salivary glands. The study of *Plasmodium* refractoriness is important for understanding transmission dynamics, and is relevant within the molecular focus of control. This is the first time that this phenomenon has been reported in vector populations from Venezuela.

### Molecular characterization of the esterase B haplotype involved on insecticide resistance in *Culex quinquefasciatus* from Cuba

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Overproduction of nonspecific esterase enzymes due to gene amplification is a common mechanism of insecticide resistance in mosquitoes. Esterases are attributed to closely linked loci called est-2 (or est-B) and est-3 (or est-A). In resistant mosquitoes, a few amplified haplotypes (e.g., B1, A2-B2) have been found to be widely distributed throughout the world. In susceptible mosquitoes, an extensive polymorphism has been found at the esterase B locus. These findings have led to a hypothesis of migration of resistance genes. We characterized this phenomenon using restriction fragment length polymorphism with *EcoRI* in single mosquitoes, and restriction mapping (double digestion method, using 10 endonucleases: *BamHI* [B], *BglII* [G], *Bsp1286I* [P], *EcoRI* [E], *HaeIII* [A], *HindIII* [H], *HinfI* [F], *SalI* [S], *SmaI* [M], *XhoI* [X]) of the amplified B haplotype in 2 resistant Cuban *Culex quinquefasciatus* Say strains collected in Santiago de Cuba (Stgo) and Havana City (Quibú), 2 cities lo-

cated about 900 km of one another. We detected the same B1-like amplified haplotype in our mosquitoes, which both differ in one *Hind*III restriction site when compared with the B1 haplotype of the reference strain, TemR (California).

These results reflect the involvement of gene migration in insecticide resistance in Cuba. Because identical haplotypes were found in both Cuban cities and the appearance of a new amplified haplotype, although very similar to the reference one, is suggested, we were able to detect at least one difference located in the region that is coamplified with the gene itself.

#### **Microtiter assay for determination of glutathione-S-transferase in *Culex quinquefasciatus* strains from Cuba and Venezuela**

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The 3 principal routes of xenobiotic detoxification in animals and plants are enzymatic hydrolysis, oxidation, and conjugation of intrusive compounds into less toxic, more water-soluble metabolites. In insects, the enzymes responsible for these reactions become mechanisms for insecticide resistance when they are present in higher than normal amounts or have higher affinity for the poison. Glutathione-S-transferases (GSTs) are a family of enzymes that catalyze the conjugation of glutathione to a variety of electrophilic compounds. Several studies have correlated resistance to insecticides with increased levels of GST activity and the production of different isoforms. In the present work, we standardized a microtiter assay for determination of specific activity of GST using a *Culex quinquefasciatus* Say strain selected with the pyrethroid insecticide lambda-cyhalothrin for 6 generations (SP6). The saturation values for reduced glutathione and 1-chloro-2,4-dinitrobenzene were 19.04 mM and 2.38 mM, respectively. We also determined that 3 min was enough time to differentiate between the resistant strain, SP6, and the susceptible strain, S-Lab ( $P > 0.05$ ). After crossing the SP6 and S-Lab strains, we found that GST was inherited as a dominant character. Additionally, 8 *Culex* strains from Cuba and Venezuela were tested for GST activity. Miranda, a strain from Venezuela, showed the highest activity for this detoxifying enzyme. Values were compared with nonspecific esterase and altered acetylcholinesterase frequencies. Relationships between GST activity and resistance to different insecticides were also assessed. This is the first time that the GST mechanism was detected in *Cx. quinquefasciatus* mosquitoes from Cuba and Latin America.

#### **Species of *Lutzomyia* as possible vectors of leishmaniasis in the city of Bucaramanga, Santander, Colombia**

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One hundred twenty-three urban cases of cutaneous leishmaniasis were reported in the city of Bucaramanga in the last 5 years. This study was carried out to determine the species of *Lutzomyia* present in the city and their role in public health. The search for sand flies was made in 8 zones of the city from September to December 1996 using Centers for Disease Control (CDC) light traps and direct aspiration in resting sites and on protected human bait. Two hundred twelve specimens of 11 species of *Lutzomyia* and one species of *Brumptomyia* were collected. Among the anthrophillic species found, *Lutzomyia ovallesi* (Ortiz) and *Lutzomyia gomezi* (Nitzolesco) are known vectors of *Leishmania braziliensis*. *Lutzomyia ovallesi* had the greatest distribution over the city and *L. gomezi* was the species most frequently captured with human bait. Both species were found in the sectors of the city where most cases of leishmaniasis were reported. The finding of these species in the city suggests the possibility of infections occurring within the urban area and the need to pursue studies to break the transmission cycle there.

#### **Biological parameters of *Lutzomyia shannoni* (Diptera: Psychodidae) under experimental conditions**

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*Lutzomyia shannoni* Theodor is the vector of vesicular stomatitis in the United States and a suspected vector of leishmaniasis in Colombia. To determine factors that might affect growth in a colony of *L. shannoni* established in the Laboratory of Entomology in the National Institute of Health in Bogota, biological development was studied in 2 experimental conditions: in polystyrene boxes at room temperature and in an incubator (26–27°C). No significant differences were obtained in the speed of development of eggs, 1st-instar larvae, and adults, but other instars did show significant differences. Based on female cohorts, the following population parameters were calculated: net reproductive rate,

generational time, intrinsic rate of increase, and reproductive value. Survivorship tables were prepared and the mortality increments were described for each instar. Total egg loss was 91.2% at room temperature and 67.1% in the incubator, due to death of females, retained eggs, sterile eggs, and embryonic death. This analysis provides useful parameters for handling colonies of these insects in the laboratory and determining causes of mortality.

**Operational experience on the use of two formulations of *Bacillus sphaericus* (strain 2632) in different types of breeding sites of *Anopheles albimanus* and *Culex quinquefasciatus* in Honduras**

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During April–August 1997, 2 formulations of *Bacillus sphaericus*, strain 2632, (Griselesf) at a rate of 10 ml/m<sup>2</sup> were tested for the field control of *Anopheles albimanus* Wiedemann and *Culex quinquefasciatus* Say in the area of El Progreso, Honduras. Both species were susceptible to *Bacillus sphaericus*. Data were analyzed to calculate the percent reduction of larval indicators. Environmental conditions did not permit monitoring of the *Culex* breeding sites longer than 10 days. *Anopheles*

breeding sites were monitored for up to 90 days. Breeding sites included fish farms, cattle drinking troughs, and drums. Both formulations achieved satisfactory control of *An. albimanus* for up to 90 days. Noticeable differences were found in length of control between the 2 formulations and between different types of breeding sites.

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