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MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A FIFTH SYMPOSIUM[1]

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ABSTRACT. The fifth Spanish language symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 61st Annual Meeting in Portland, OR, in March 1995. The principal objective, as for the previous 4 symposia, was to increase and stimulate greater participation in the AMCA by vector control specialists, public health workers, and academicians from Latin America. This publication includes summaries of 20 presentations that were given in Spanish by participants from 6 countries in Latin America and the USA. The symposium included the following topics: ecological and genetic studies of anopheline vectors of malaria, laboratory and field evaluation of chemical and biological control agents for several mosquito species, and community control of *Aedes aegypti.*

INTRODUCTION

The American Mosquito Control Association (AMCA) is the world's leading professional organization dedicated to the study and control of mosquitoes and other vectors. The AMCA promotes closer cooperation among those directly or indirectly concerned with or interested in mosquito control and related work. Toward that end and to further enhance the international role of the association, a Spanish language symposium was held in 1995 at the 61st Annual Meeting in Portland, OR. This marked the fifth consecutive year in which this session was held. Twenty presentations were given in Spanish by participants from 6 countries in Latin America and the USA. The symposium included the following topics: ecological and genetic studies of anopheline vectors of malaria, laboratory and field evaluation of chemical and biological control agents for several mosquito species, and community control of *Aedes aegypti.* Summaries of 3 previous symposia have been published (Clark and Suarez 1991, 1992, 1993).

Financial support for this unique and innovative session at the Annual Meeting continues to be good. Recognition for financial support for the 1995 symposium goes to the following sponsors and individuals: Clarke Mosquito Control Products (John L. Clarke, Jr.); ZENECA (Eduardo Moreira); ADAPCO (Allen Wooldridge); Northeast Vector Management (Bill Zawicki); Beecomist Systems (Ed Kutzner); Vectec, Inc. (Ed Meehan); American Cyanamid (Bill Jany); Summit Chemical (Larry Kase); ARRO-GUN Spray Systems (Dan Ariaz); Zanus Corporation (David Sullivan); JAYCO Products (Jay Stewart); and the Northwest Mosquito Vector Control Association (Peter DeChant). Special recognition also goes to the Pan American Health Organization (Dr. Gabriel Schmunis and Dr. Michael Nelson) for providing funds that supported attendance of several speakers. Special thanks go to Dr. Ronald A. Ward, Editor of the JAMCA, for his assistance and encouragement to publish these summaries and for identifying funds for this publication.

As a result of the positive and enthusiastic response from participants and the AMCA leadership, this unique forum will be included in future meetings. In this way, the problems caused by mosquitoes and other vectors in areas outside of the continental United States receive additional attention. Offering this symposium has served to significantly increase AMCA membership in Latin America and has fostered and facilitated increased academic, intergovernmental, and commercial interaction among colleagues living in Latin American countries and with their peers in the USA.

SUMMARIES

Larval ecology of the exotic dengue vector, *Aedes albopictus* in two states in northeastern Mexico

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An Asian vector of dengue, *Aedes albopictus* (Skuse), was first reported in the United States (Houston, TX) in 1985. In 1988, it was identified across the Mexican border from Texas in Tamaulipas State, in northeastern Mexico. The mosquito was also found in abandoned tires in

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Matamoros city adjacent to Brownsville, TX. During 1993, the vector was also reported in 2 bordering cities in Coahuila State: Piedras Negras (adjacent to Eagle Pass, TX) and in Acuña city (Del Río, TX). These reports pointed toward discarded tires as the main site for *Ae. albopictus* breeding in Mexico. However, considering the more abundant availability of breeding sites provided by typical Latin American villages and towns, plus an expected opportunistic behavior in oviposition habits of *Ae. albopictus,* objectives of this study were: 1) to determine type and frequency of breeding sites used by *Ae. albopictus* in northeastern Mexico, and 2) to measure the degree of invasion of *Ae. albopictus* over known *Aedes aegypti* (Linn.) breeding sites in the same area. A survey for *Ae. albopictus* was carried out in Tamaulipas, Nuevo León, and Coahuila states in northeastern Mexico. The search covered cemeteries, abandoned tires, and peridomestic artificial containers. Larvae were collected and reared to adult stage for identification. From April to June 1994, no *Ae. albopictus* were found in 14 villages in Nuevo León State. In November, 50 breeding sites were surveyed in Matamoros, Tamaulipas State; 92% were infested by the Asian mosquito. These peridomestic containers were 5-gallon buckets, cement tanks, 55-gallon drums, animal drinking water containers, and trays. These sites were inside houses or backyards (38%), in flower vases in cemeteries (50%), in public parks (2%), and in used car establishments (2%). This species was identified in 16% of flower vases surveyed within a private city cemetery in Piedras Negras, Coahuila State. Cemetery flower vases (8%) were also the only breeding sites in Acuña city in Coahuila. Colonization of typical *Ae. aegypti* breeding sites by *Ae. albopictus* was only recorded in Matamoros city, where this species has heavily infested urban and rural areas.

Temporal abundance of *Anopheles aquasalis* larvae in a malarious area in northern Venezuela and associated factors

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Anopheles aquasalis Curry is the principal vector of malaria in northeastern Venezuela. A study of the ecology of this species was carried out in the southern region of Paria, a coastal area of Sucre State where malaria is an important public health problem. Two hypotheses were tested: 1) the anopheline larval population has temporal and spatial nonrandom distributions, and 2) these distributions are influenced by habitat variables that can be readily identified. *Anopheles aquasalis* larvae were sampled monthly with a standard dipper for a 1-year period (July 1993–June 1994) in 4 dominant habitat types (brackish: mangrove lagoon; freshwater: marsh, pond, and swamp), and population density (larvae/dip) was calculated from 30 dips. Twelve selected aquatic variables were quantified (vegetation cover, plant height, phytoplankton, associated aquatic organisms, water depth, pH, temperature, dissolved oxygen and carbon dioxide, conductivity, salinity, and alkalinity) to describe larval habitat and predict temporal occurrence of the species. Temporary shallow mangrove lagoons consistently harbored much higher densities of *An. aquasalis* (9.15 ± 1.12/dip) than permanent freshwater ponds (1.0 ± 0.2/dip) and shallow marshes (0.9 ± 0.1/dip), whereas semipermanent swamp habitats contained no larvae. Conductivity, salinity, and phytoplankton were significantly higher, and alkalinity and animal species richness were lower in high-density *An. aquasalis* sites than in low-density sites. Higher population density occurred at the end of the rainy season (September–October), with no correlation found between density and rainfall. Regression analysis between density and habitat variables showed a positive correlation with (in order of importance) salinity, depth, aquatic plant height, and dissolved oxygen; and a negative association with pH. These variables explained 80% of the variation in larval density. The breeding of *An. aquasalis* in high-salinity habitats was confirmed by our results; the results also revealed that the majority of environmental factors evaluated, specially salinity and depth, can be good predictors for the species' reduced temporal distribution. The results suggest the feasibility of formulating appropriate control strategies for *An. aquasalis* in northern Venezuela. This research was funded by CONICIT grant RPIV-130032-11.

Vector biology of resting populations and biting biorhythm of *Culex tarsalis* populations in Monterrey, northeastern Mexico

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Studies of potential vectors of equine encephalitis are lacking in Mexico. Despite frequent reports of arbovirus activity in several states in the

US along the Mexico–USA border, knowledge of viruses in mosquito vectors is poorly documented in Mexico since the Venezuelan equine encephalitis (VEE) outbreak in the 1970s. *Culex tarsalis* Coq., a VEE and eastern equine encephalitis (EEE) virus vector, is abundant in rural and urban areas of Monterrey in northeastern Mexico. We studied age-structure, insemination rate, trophic and physiological stages, and biting rhythm of resting females *Cx. tarsalis*. The study site was in an agricultural area 20 km from Monterrey. Resting mosquitoes were collected in 23 red boxes (1 × 2 × 1 ft.) and 5 old tires (5-ft. diam). Eight-hundred seventy females were collected during the 20 days of the study. Mosquitoes were dissected in the laboratory and the following parameters were checked: abdominal appearance (Sella stages), parity (Detinova technique), follicular development on ovaries and ovarioles (Christophers' stages), and spermatheca for insemination. Preliminary results demonstrated that 65% (566) of the females were unfed, 15.5% (135) were bloodfed, and 19.5% (169) were gravid. Parity rate for unfed females was 21% (119), whereas 18.5% (25) was recorded for fed mosquitoes. Ovarian development showed that 89% of unfed females had ovarioles at Christophers' 1st stage, whereas Christophers' 3rd stage was found in 38.5% of fed females. Ovariole relics were recorded in 1.8%. The insemination rate was 84%. We found that most of the unfed nullipars, showing Christophers' 1st stage, corresponded to newly emerged females from nearby breeding sites. Biting rhythm was determined by human landing collections and by using a screen trap baited with a horse during a 10-day period, in a collecting schedule of 12 h from 1800 to 0600 h. A total of 400 *Cx. tarsalis* females were caught with both methods; the highest biting activity was recorded 1 h after sunset. Species collected, other than *Cx. tarsalis*, were *Aedes dorsalis* (Meigen), *Aedes triseriatus* (Say), *Aedes taeniorhynchus* (Wied.), and *Culiseta inornata* (Williston). Because all of these also have been reported as encephalitis vectors, the need to increase research on encephalitis vectors in northern Mexico is reinforced.

Dispersion of larval stages of *Anopheles nuneztovari* in fish ponds from Buenaventura, Colombia

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Anopheles nuneztovari Gabaldon is one of the primary vectors of malaria in Colombia. In Buenaventura, fish ponds are the principal breeding sites. In order to understand which factors might be useful as interventions for future control strategies, we determined the dispersion of *An. nuneztovari* larvae in fish ponds, associated with depth, turbidity, water–vegetation interphase area, orthophosphates, and dissolved oxygen. Seven fish ponds were sampled during 1993 using a 350-ml dipper; 4 ponds were surveyed 3 times. The *An. nuneztovari* larvae were counted and the instars present were determined. The dispersion of the larval population was analyzed using Taylor's power law, Lloyd's mean crowding index, and Iwao's contagious regression. The association between larval density and the habitat variables was analyzed with linear and multiple regression.

Multiple regression analysis showed that turbidity and the water-vegetation interphase area ($Y = 0.57 + 0.06veg. - 0.016turb.$; $r = 0.87$; $P < 0.001$) were important to the dispersion and abundance of *An. nuneztovari* larvae. When turbidity increased, larval food production decreased and affected larval development. This is compensated for by the microflora associated with the water–vegetation interphase. When the water level decreased, the water–vegetation interphase area diminished and larval density also decreased. Water depth accounted for 50% of the variation in the reduction in larval density.

Taylor's power law showed that *An. nuneztovari* larvae had an aggregated distribution ($b = 1.35$), but according to the alpha parameter (0.48) of Iwao's regression, this distribution was not an inherent behavior of the larval stages. The beta parameter (1.30) of Iwao's regression was low and, therefore, showed no clear relationship between aggregated distribution and habitat heterogeneity given by the extension of the water–vegetation interphase. This index and the strong relationship between Lloyd's mean crowding index and the density ($r = 0.996$; $P < 0.0001$) suggested that aggregated distribution of larval *An. nuneztovari* was determined by female ovipositional behavior and the later larval mortality.

Mosquitoes and bromeliads: species-specific selectivity patterns on the northern coast and southern Guiana Shields in Venezuela

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Collections were made at 4 locations in 3 national parks in Venezuela; two on the northern coast of Venezuela: Cerro Santa Ana (CSA) (Paraguaná Peninsula, near the Lesser Antilles) and Sierra de San Luis (SSL) (continental origin

close to coastal Andean mountains); and two on the south Guiana Shield: La Gran Sabana (LGS) and Auyan-Tepui (AUY) (western Tepui summit). Thirteen mosquito species were identified in 15 Bromeliaceae species and one species of Sarraceniaceae. The mosquito species richness collected in the plants per location (mosquito species/plant species) showed the following order: LGS (9/5), SSL (8/5), AUY (5/6), and CSA (4/6). The "Guild Structure" analysis established a selection model (D) for the bromeliad resource and a resource selection index (R) for the most selected plant species. Selectivity (positive and significant) by one or more plants species was high in SSL with 6 of 8 mosquito species (75%); medium in Guiana Shield: LGS (55%) and AUY (50%); and low in CSA (25%) [D > Z (0.99) = 3.82].

The mosquito species with the greatest preference for plant species ["D" > Z (0.99) = 3.82] per location were *Aedes sexlineatus* (Theobald) and *Wyeomyia bicornis* (Root) in SSL; *Wyeomyia fishi* Zavortink, *Aedes* (*Howardina*) sp. nov., and *Wyeomyia* (*Cruzmyia*) sp. nov. in LGS; *Runchomyia frontosa* (Theobald) and *Anopheles* (*Kerteszia*) "Auyantepui Mesa form" in AUY; and only *Wyeomyia celaenocephala* Dyar and Knab in CSA. The most selected plants [R > Z (0.99) = 3.82) were *Vriesea platynema* Gaudichaud and *Aechmea paniculigera* (Swartz) in SSL; *Brocchinia micrantha* (Baker) and *Brocchinia reducta* Baker in LGS; *B. hectioides* Mez and *V. rubra* (Ruiz and Pavon) in AUY; and *Guzmania lingulata* (Linn.) in CSA.

A species-specific selectivity pattern was observed in some mosquito species (49% of the species did not show selectivity) and this pattern depends on factors such as: degree of isolation and geoevolutive origin in the location, bromeliad resource abundance, macroclimatic factors as consequences of altitude, and microclimatic factors associated with the plant itself. Support for this research was provided by CONICIT RP-VII-240060 and PAHO.

Evaluation of light traps combined with carbon dioxide and 1-octen-3-ol to collect anophelines in Venezuela

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In a search for a satisfactory method for sampling anophelines that would reduce the need to use human landing catches for routine evaluation of control programs, updraft ultraviolet light traps combined with carbon dioxide (CO_2) and 1-octen-3-ol (octenol) were compared with the CDC light trap and human landing catches of *Anopheles albimanus* Wied. and *Anopheles aquasalis* Curry.

The study was carried out in Puerta Negra (10°06'N, 67°36'W), 20 km south of Maracay, Aragua State, in northcentral Venezuela. Collections were carried out between 1830 and 2130 h, 3 nights/wk every month between September 1994 and February 1995. Six collection stations located 100 m apart were established forming a cross to test the following methods: 1) landing catches on 2 human baits; 2) a battery-operated, updraft UV light trap; 3) an updraft UV light trap plus CO_2; 4) an updraft UV light trap plus octenol; 5) an updraft UV light trap plus octenol and CO_2, and 6) the CDC light trap. Each method was rotated each night so that equal number of collections with each method were carried out at each station. The following morning mosquitoes were killed, identified, counted, and dissected for parity.

Preliminary results show that significantly more *An. albimanus* were collected with the updraft UV light trap alone or with any of the attractants than *An. aquasalis*. The CDC light trap used alone was inefficient in collecting these species. The updraft UV light trap was more efficient for catching *An. albimanus* when used with octenol, whereas the use of octenol plus CO_2 did not significantly increase the number of *An. albimanus* collected. About 20% of the anophelines caught in all light traps could not be identified. Of the anophelines caught in the CDC light trap, 75.7% were males; 30.3% males were caught in the updraft UV light; whereas the use of attractants produced only 4% males for octenol and 9% males for CO_2 and CO_2 plus octenol. The updraft UV light trap used with octenol as an attractant is a promising collection method that can substitute for or complement human landing catches for monitoring populations of *An. albimanus* but not *An. aquasalis*.

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Investigations of detoxifying esterases in *Culex pipiens quinquefasciatus* from Martinique and surrounding countries

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Several over-produced esterases confer resistance to organophosphorus insecticides in the *Culex pipiens* complex. Our preliminary investigations showed that 4 esterases of high activity (esterase B1, the associated esterases A2–B2, and esterase C2) have a wide geographic distribution and frequency in the Caribbean area: Cuba, Puerto Rico, Haiti, St. Martin, Guadeloupe, Martinique, St. Lucia, Barbados, French Guiana, and Venezuela. These results underscore the importance of indirect insecticide pressure and/or passive resistance on gene migration, because in many countries there is no general vector control program against *Culex pipiens quinquefasciatus* Say.

Linkage disequilibrium in elevated esterases of *Culex quinquefasciatus* in the presence and absence of insecticide selection pressure

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In *Culex quinquefasciatus* Say, the elevated esterases B1, B2, and A2 are involved in broad spectrum organophosphate insecticide resistance. The B1 esterase has a limited distribution, with reports of its presence only in California and Cuba. We now report the occurrence of B1 in 2 additional field populations from Trinidad and Colombia. In both locations, as well as in Cuba, 2 more elevated esterases, A6 and B6, have also been found. Previously, the A2 and B2 esterases had been found in numerous field populations of *Cx. quinquefasciatus* where the A2, B1, and B2 esterases are all present. The A2 and B2 esterases no longer show any evidence of linkage disequilibrium in the absence of positive insecticide selection pressure.

Population genetic analysis of *Anopheles aquasalis* in Venezuela and Trinidad

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An outbreak of *Plasmodium vivax* malaria occurred in Icacos, Trinidad, in 1991 and was then traced to a Trinidad fisherman who had visited Pedernales (Delta Amacuro State), Venezuela. The vector of malaria in both countries is *Anopheles aquasalis* Curry. Continuous human migration between Venezuela and Trinidad and the presence of different morphologic characteristics in *An. aquasalis* populations from Pedernales established a need to determine the genetic structure of both populations for risk assessment. To do this, 6 populations from the west coast of Trinidad and one population from Pedernales were analyzed for genetic variation at 18 isozyme loci. Estimates of genetic variability for *An. aquasalis* from Pedernales were relatively larger than the values obtained in Trinidad. The percentages of polymorphic loci were 22–33%; the average heterozygosities were 0.125 and 0.08, respectively; and the mean number of alleles per locus (1.9) was the same for both populations. The value of F_{ST} (Wright's F statistics), averaged over all loci, was 0.0516 indicating that there is only minor heterogeneity in allelic frequencies, but there were positive values of Wright's fixation index for 3 enzyme-electromorph loci in each country. These results indicate the need for local vector control to reduce disease incidence. Support for this research was provided by PAHO; CONICIT RPIV-130032; Ministry of Health, Venezuela; and Ministry of Health, Trinidad, W.I.

Polymorphism of circumsporozoite protein of *Plasmodium vivax* from Colombia

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Malaria infection begins when infected *Anopheles* mosquitoes inject sporozoites into the blood vessels. The circumsporozoite protein (CS) is the most extensively studied among several proteins that have been identified on the surface of the sporozoites. The *Plasmodium vivax* CS protein contains a central region composed of multiple tandem repeats of a nonapeptide. According to the gene sequences, this region presents 2 variants, designated type 1 or "common" and type 2 or "variant," that can be differentiated using monoclonal antibodies (mAb). These mAb have proven to be useful in the detection of sporozoites in salivary glands of infected mosquitoes. In addition, due to its immunogenicity, this region has been incorporated into synthetic and recombinant malaria vaccine candidates.

In order to determine the extent of polymor-

phism of the repetitive region of the CS in isolates of *P. vivax* from Colombia, sporozoites were obtained by experimental infection of *Anopheles albimanus* Wied. Mosquitoes were fed by artificial membrane on infected blood of 38 individuals from different malaria endemic areas and sporozoites were recovered 14 days after infection. Thirty-eight isolates were analyzed using an immunofluorescence test with 2 mAb specific for both type 1 or type 2 of the *P. vivax* CS. The type-2 sequence was found in 21 isolates (55%), and the type-1 sequence in 13 (34%) isolates. Three (8%) of the isolates reacted with both mAb, and one was negative for both mAb. The high frequency of the type-2 sequence among the Colombian isolates indicates the necessity to use both mAb to accurately determine the infection of malaria vaccine candidates. Support for this research was provided by COLCIENCIAS and a grant from the Commission of the European communities.

Revised list of the mosquitoes of Mexico

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The serious study of the mosquito fauna of Mexico started with the original lists published in 1956. A revised list of the anopheline mosquitoes was recorded in 1976 and a more extensive list of 118 species was given in 1977. Many species have been added to the fauna, there are also many supraspecific changes to report.

A concentrated effort to know the Mexican mosquitoes was made in the 1940s to 1960s due to the important work of Dr. L. Vargas and his colleagues. Many species were added in the late 1960s and the 1970s by Dr. J. N. Belkin and his students who were involved in the project "Mosquitoes of Middle America." Until now, no attempt had been made to assemble a complete list of the Mexican mosquito fauna. An accompanying and necessary parallel is the compilation of a bibliography dealing with Mexican mosquitoes. To date, 142 titles are included in such a list of relevant references.

At present 20 genera, 37 subgenera, and 223 species are known in the Mexican mosquito fauna (Table 1). Many details of the list will be discussed in a more thorough report later, but 2 issues are emphasized here. The following species have been erroneously reported from Mexico: *Aedes impiger* (Walker), *Aedes punctor* (Kirby), *Aedes fulvithorax* Lutz, *Culiseta impatiens* (Walker), *Deinocerites epitedeus* (Knab), and *Deinocerites spanius* (Dyar and Knab). Also, there are 29 species that are apparently restricted to Mexico, based on present knowledge.

Table 1. Genera of mosquitoes occurring in Mexico and the number of subgenera and species in each.

Genus	No. subgenera	No. species
Aedeomyia	1	1
Aedes	7	60
Anopheles	3	26
Chagasia	0	1
Coquillettidia	2	4
Culex	9	60
Culiseta	1	3
Deinocerites	0	6
Haemagogus	1	4
Johnbelkinia	0	1
Limatus	0	1
Mansonia	1	2
Orthopodomyia	0	1
Psorophora	3	18
Sabethes	2	4
Shannoniana	0	2
Toxorhynchites	1	4
Trichoprosopon	1	2
Uranotaenia	2	9
Wyeomyia	3	14
Totals	37	223

Susceptibility of *Anopheles darlingi* to chemical insecticides in Bolivar State, Venezuela

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The susceptibility to chemical insecticides was evaluated in 2 populations of *Anopheles darlingi* Root from Bolivar State, where malaria transmission due to this species represents a high percentage of malaria cases reported for Venezuela. The standardized methods at the diagnostic doses of the World Health Organization were used in this study. The results were similar in both populations, with a high level of tolerance to DDT and cypermethrin; a low level of tolerance was found to the following insecticides—organophosphate: malathion, fenthion, pirimiphos methyl, and fenitrothion; carbamates:

propoxur and bendiocarb; and synthetic pyrethroids: lamdacyhalothrin, deltamethrin, and cyfluthrin. These results will permit a better implementation of the chemical control strategy used for *An. darlingi* through a rational program of insecticide rotation in order to maintain low levels of tolerance to the most commonly used insecticides.

Residual effect of 1% Abate® granules and 5% Abate® pellets in field and laboratory conditions in Monterrey, northeastern Mexico

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The larvicide Abate® (temephos 1% granules) is intensively used by the Vector Control Program in Mexico against immature populations of the dengue vector, *Aedes aegypti* (Linn.). A concentration of 1 part per million (ppm) per liter of water is used in mosquito breeding sites. However, water management, washing, and refilling of peridomestic containers such as drums, buckets, and cement tanks affects residuality of the temephos granular formulation. During this research the aims were: 1) to compare residuality and effectiveness of the 1% granular Abate formulation versus the 5% Abate pellets in the same concentration of 1 ppm/liter, a larvicide already in use in the United States for reducing larval populations of *Ae. aegypti* and *Culex quinquefasciatus* Say breeding in domestic 55-gallon drums; 2) to compare alternative doses of the 5% Abate pellets such as 2, 3, 4, and 5 ppm/liter in both residuality and efficacy; and 3) to determine the same parameters under laboratory conditions. Field trials were conducted in a suburban residential area near Monterrey. A group of 35 drums belonging to residents was treated with the larvicide as follows: 1 ppm/liter of 1% granular Abate; 1 ppm/liter of 5% Abate pellets; 2, 3, 4, and 5 ppm/liter of 5% Abate pellets (5 drums were used for each dose); and 5 drums that served as controls. People were allowed to use the water as they routinely do to determine the effect of such activities on larvicide efficacy. Comparisons were made with another group of 35 drums containing the same dosages but without permitting people to use the water. Drums and dosages were randomly selected for the dwellings. Samples of dead and live larvae were collected fortnightly from October 1993 to May 1994. The laboratory study was made using 55-gallon drums treated similarly but using only one drum per dose. Drums were set for 8 months; assays were made every 2 wk using 300 ml of treated water with 25 *Ae. aegypti* larvae. Mortality was recorded after 24-h exposure. Field results showed an overall 100% mortality for 16 wk for all doses regardless if water was used or not. Abate 5% pellets at 3 and 5 ppm/liter produced the highest residual effect, 24 and 27 wk, respectively. Due to severe lack of water supply during the study interval, people used water in several drums without permission. This fact limited our results from both formulations and concentrations in the field. This practice emphasizes the need to involve participation of the community to increase vector control success even when chemical control is utilized. The last statement is supported by our finding of 100% mortality and 8-months residuality in the laboratory study for all concentrations and formulations tested.

Susceptibility studies of *Aedes aegypti* from different regions of Venezuela to organophosphorus and pyrethroid insecticides

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The basic tool for the control of *Aedes aegypti* (Linn.), the principal vector of dengue and dengue hemorrhagic fever (DHF) in Venezuela, has been the use of chemical insecticides. This strategy when used for several years can select vector populations more tolerant to and eventually resistant to some insecticides. For this reason, the objective of this research was to establish the baseline of susceptibility to organophosphate and pyrethroid insecticides for *Ae. aegypti* larvae from different states of Venezuela (Araguay, Apúre, Barinas, Carabobo, Guárico, Lara, Miranda, and Táchira) where dengue and DHF transmission has been high. World Health Organization procedures were used.

Preliminary results indicated that 6 out of 8 field strains were susceptible to malathion (Resistance Factor 0.50 < 3) and the other 2 reached a verification level with a Resistance Factor 0.50 between 5.8 and 7.8. All field strains were susceptible to fenitrothion and tolerant to pirimiphos methyl. As for the synthetic pyrethroids, we found deltamethrin resistance without any cross-resistance to lamdacyhalothrin, cypermethrin, or cyfluthrin. These results will permit the prediction of the levels of resistance in the adult stage.

Field effectiveness of three insecticides in ULV spraying of malathion, Dursban and Mosquito Master 412 against *Aedes aegypti* and *Culex quinquefasciatus* in Monterrey, Mexico

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In many Latin American countries, emergency control measures during outbreaks of dengue fever and malaria are based mainly on ultralow volume insecticide spraying. For decades, malathion (95%) has been the most used adulticide to reduce adult densities. Clearly, the search for alternative chemicals is needed in developing countries interested in controlling these diseases. This study was conducted to determine field efficacy of the commonly used malathion 95% and 2 other chemicals that have never been used in Mexico—Dursban (chlorpyrifos 12%) and Mosquito Master (chlorpyrifos 12% plus 4% permethrin)—to kill caged adult populations of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say. Tests were conducted in an open, vegetated area in a semiurban location 10 km from Monterrey. An LECO 500 aerosol generator was used, at the following insecticide flow rates: 6.3 oz./min for each chemical and a second rate of 6.5 oz./min for Dursban. Open and vegetated assays were conducted by placing 2 parallel, 600-ft.-long lines of caged mosquitoes. Cages for each line were placed at 6 different exposure distances: 100, 200, 300, 400, 500, and 600 ft. A total of 24 cages, each containing 20 female mosquitoes, was hung at a height of 6 ft. Sprays were made separately for each species and for open and vegetated areas in the study site. Vehicles carrying the generator drove at 10 mph; each assay was carried out at sunset while recording ambient temperature, wind velocity, and direction. Controls were placed during all field tests. The effectiveness of insecticides was recorded as 1-h mortality. Greater than 85% mortality was produced by each chemical for both mosquito species up to 300-ft. distances in the open area. A 100% 24-h mortality was recorded for all chemicals in 6 tested distances in the vegetated area. The combined product Mosquito Master showed a higher 1-h mortality effect. Mortality was reduced 4.8% for every 100-ft. distance in the open area. A 5.6% mortality reduction for every 100 ft. was also recorded for the 3 chemicals in the open area at 24 hr. The study also addressed costs and benefits of traditionally used malathion versus Dursban and Mosquito Master 412, an important consideration in Latin American countries wanting to replace malathion.

Utilization of the copepod *Mesocyclops longisetus* (Copepoda: Ciclopoidea) to control larval populations of *Aedes aegypti* in domestic cement tanks in Chiapas, southeastern Mexico

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Recent work has considered the possibility of using predacious copepods to reduce immature populations of the dengue vector *Aedes aegypti* (Linn.). Satisfactory results have been obtained under controlled conditions and also in field situations where water is always present in breeding sites. However, successful use of copepods released in the field is highly dependent on community participation. Cement tanks (pilas) represent the chief breeding site for the dengue vector in villages in southeastern Mexico. They maintain water as well as larval populations of mosquitoes throughout the year. This work was aimed at evaluating the efficiency of the copepod *Mesocyclops longisetus* (Thiébaud) in reducing larval populations of *Ae. aegypti* in pilas in Huixtla, a city with 30,000 residents near Tapachula in southeastern Mexico.

Additional objectives were to assess copepod survivorship when assisted by community participation and to assess predator reproduction rate in pilas. Research was conducted during the rainy season from June to November 1994. A group of 600 pilas was selected in houses in Huixtla; 300 were treated with 200 ovigerous female *M. longisetus,* and 300 served as controls. Residents where pilas were treated received a 30-min talk on dengue, the role of *Ae. aegypti* larvae, and the copepod controlling agent. Directions on how to take care of ciclopoids at the time of pila washing were also provided in a calendar nailed on the wall of the house. A marker set in the pila bottom was also a reminder of copepod presence for residents. Fifty treated pilas and 50 controls were sampled monthly to check copepod abundance and larvae densities. Preliminary results showed that a mean of 19% (min. = 9.3, max. = 27) of the pilas maintained copepod populations during the 6-month study. *Mesocyclops longisetus* was not able to successfully reproduce in these containers; of 200 females initially introduced per pila, only a mean of 23.2 (min. = 10.2, max. = 38.7) copepods was present 6 months later. Comparisons of larval densities between treated and control pilas did not show statistical differences.

Likely explanations for these results may be attributed to poor community participation, frequent washing of the pilas, and drainage of the water through a hole in the bottom of the pila.

Field evaluation of *Mesocyclops longisetus* (Copepoda: Ciclopoidea) in controlling larval populations of *Aedes aegypti* in Monterrey, Mexico

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Mesocyclops longisetus (Thiébaud) has shown potential as a biocontrol agent for reducing larval populations of the dengue vector *Aedes aegypti* (Linn.). Field tests in Honduras proved the effectiveness of *M. longisetus* in peridomestic breeding sites; however, considering differences in breeding sites and in general larval ecology from a tropical region versus a temperate area such as northeastern Mexico, effectiveness of ciclopoids could vary. Therefore, this study was aimed at determining reduction in mosquito larval density by copepods and copepod survivorship when assisted by community participation in breeding sites of epidemiological importance in Monterrey, Mexico. Field tests were conducted in Colonia El Mirador, San Nicolas de los Garza, Nueva Leon, Mexico. Two hundred ovigerous *M. longisetus* females were introduced in each of 60 55-gallon drums in several dwellings. In 40 of these drums, people were allowed to use water as they ordinarily did. Also, a marker was placed in the bottom to remind residents that they were taking care of copepods during drum washing. In the remaining 20 drums, water use was not permitted by the residents in order to determine the survivorship rate of *M. longisetus* in this aquatic environment. Controls were included for both of these groups. At the same time, 50 abandoned tires in backyards were each treated with 50 ovigerous female *M. longisetus* and 20 tires served as untreated controls. A 3rd breeding site that was included was flower vases in the city cemetery. Fifty flower vases were each treated with 50 female copepods and 30 were used as controls. Fortnightly sampling of only larval populations occurred in all breeding sites from May to September 1994. At the end of the study, copepods were removed and counted to assess survivorship, reproduction rate, and larval control. After 4 months, copepods were present in 90% of the drums where water was intensively used. The success of this program was supported by community participation. However, larval reduction was only 37.5% in drums where people utilized the water. A similar reduction (39.9%) of dengue vector populations was found in drums where people did not use the water. Many (54%) of the backyard tires maintained copepod populations during almost 4 months, but larval reduction corresponded only to 40.9%. A greater effectiveness of *M. longisetus* was found in cemetery flower vases, where they reduced larval populations up to 67% and survived in 90% of treated vases during a 4-month period. Reproduction of ciclopoids in flower vases produced a mean of 450 copepods per vase, about 9 times more than initially introduced populations. However, during the cemetery study, water had to be poured into vases every week because local precipitation in this semiarid region was scarce. Our results indicate that use of copepods to control *Ae. aegypti* populations may be of limited value in places with a short rainy season and with a poor nutritional environment for copepods in metal drums.

Preliminary evaluation of *Vavraia* sp. (Microsporidia: Pleistophoridae) on *Culex quinquefasciatus* under laboratory conditions

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A microsporidian of the family Pleistophoridae, genus *Vavraia,* was found infecting mosquito larvae growing in bromeliads near the settlement of Joví (Pacific coast of Chocó, Colombia). Mosquitoes found in bromeliads belonged to the genera *Wyeomyia, Anopheles,* and *Orthopodomyia.* Natural infection of larvae by *Vavraia* was 6.8%, afflicting *Wyeomyia circumcincta* Dyar and Knab, *Wyeomyia simmsi* (Dyar and Knab), *Anopheles neivai* Howard, Dyar and Knab, and *Orthopodomyia* sp.

Laboratory-reared *Culex quinquefasciatus* Say larvae were subject to colonization by *Vavraia* sp. Its life cycle was studied in the laboratory colony, where we observed meronts with 2, 4, 8, 16, and 32 nuclei and sporonts containing sporoblasts with 8, 16, 32, and 64 spores. Spores produced in *Cx. quinquefasciatus* were egg-shaped, 4, 78 ± 0.44 μm long and 2, 66 ± 0.23 μm wide. In the pathogenicity study, we determined the effect of increasing concentrations of 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6, and 1×10^7 spores/ml on the longevity and fecundity of *Cx. quinquefasciatus.* We analyzed the results using the MANCOVA test and the Tukey significance test with a 95% confidence.

In the group of individuals that survived from the larval, pupal, and adult stages, we observed

significant differences only between the concentrations of 1×10^5 and 5×10^6 spores/ml in pupae and adults. We analyzed longevity based on the number of weeks during which 50% of the populations died and found no statistical differences. Fecundity was analyzed by observing the number of egg rafts laid weekly by females; we observed significant differences between the control group and the concentrations of 5×10^5 and 5×10^6 spores/ml. We determined the infection concentrations 50 and 90 (IC_{50} and IC_{90}), which were 24,865 and 684,026 spores/ml, respectively.

Characterization of *Aedes aegypti* breeding sites in Panama according to the population social stratum, pH, and bacterial contamination

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To characterize the breeding sites of *Aedes aegypti* (Linn.) in the metropolitan area of Panama City during 1994, 3 counties with populations having different social characteristics were chosen. These were Curundu (extreme poverty), Rio Abajo (lower and middle class), and Bella Vista (middle and upper class). The pH of the water of 102 of the most frequently found breeding containers was recorded and 50 water samples were cultivated to determine the associated microflora and levels of fecal contamination.

Results revealed the following:

1. *Aedes aegypti* breeding sites varied in accordance to the social stratum, with a definite trend toward more nonusable breeding containers in low income areas and a higher proportion of usable breeding containers associated with areas having better social conditions. However, there was a large number of potential breeding sites in areas from all social strata.
2. The most abundant *Ae. aegypti* breeding sites were of plastic, metal, and concrete in the corregimiento of Rio Abajo.
3. The pH was not a critical factor in the breeding sites, varying from 5.0 to 8.0.
4. Some bacterial indicators of fecal contamination (e.g., *Proteus, Enterobacter, Klebsiella*) were found, even though the major indicator of fecal contamination, *Echerichia coli,* was not found. We concluded that even though *Ae. aegypti* is usually found breeding in relatively clean waters, its larva can tolerate a variable degree of fecal contamination.

Dengue next door—the Panama Canal response to the 1994 epidemic in Panama

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Over the past decade, dengue fever has become more frequent throughout Latin America and the Caribbean. Following the 1985 reinfestation of *Aedes aegypti* (Linn.), this dengue vector spread throughout Panama City and to other cities and towns in the Republic of Panama. As house indices rose in nearby urban districts, the Panama Canal Commission became concerned about the possibility that a massive dengue epidemic could drastically affect the operation of the Panama Canal, as more than 80% of the Canal workforce lives in or near these areas.

On November 19, 1993, after some 50 years of being free from dengue, the Minister of Health of Panama confirmed that the first case of this disease, in a suburban development on the outskirts of Panama City, had been diagnosed in the laboratory. Fourteen cases of DEN-2 were recorded in this neighborhood before transmission ceased in early December.

Prior to the outbreak, Commission public health and vector control officials had developed a comprehensive "dengue contingency plan" that centered on education of the Panama Canal workforce about how dengue is transmitted, its symptoms, and what to do if dengue was suspected. Agency-wide classes covering these topics were presented and educational fliers and pamphlets were distributed to all employees giving "action lists" of what to do to prevent *Ae. aegypti* from breeding around the home. Community and neighborhood cleanup campaigns were emphasized in employee newspaper articles and recommendations were given on how to reduce the risk of contracting the disease. Panama Canal "larva hunter" teams were augmented to provide weekly vector surveillance, source reduction, and mosquito control within Canal residential areas, Commission facilities and buildings, as well as adjacent housing areas. Close coordination was also maintained with U.S. military health and vector control officials, as well as diagnostic laboratory personnel, epidemiologists, and vector control authorities of the Ministry of Health.

In July 1994, epidemic dengue (DEN-1) began in an urban district of Panama City and rapidly spread to other parts of the metropolitan area. Peak transmission occurred in August and September and the number of cases declined from October through December. Separate out-

breaks took place in the province of Chiriqui (near the border with Costa Rica) and in the interior province of Herrera during the last 2 months of the year. Remarkably, even though near the entire population of Panama had not been exposed to dengue for 5 decades, only about 800 cases of dengue were confirmed in the Republic during this outbreak and no cases of hemorrhagic dengue or dengue shock syndrome were observed.

To the best of our knowledge, none of the approximately 8,500 Panama Canal Commission employees or their dependents became ill with dengue during this epidemic.

Evaluation of four different interventions for *Aedes aegypti* control in Merida, Yucatan, Mexico

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During a clean-up campaign that was held to eliminate breeding sites in the city of Merida, Yucatan, 4 different educational and promotional interventions were promoted to help the community identify and eliminate those *Aedes aegypti* (Linn.) breeding sites, defined as disposable containers. These interventions were done in 4 neighborhoods with similar features to find which approach resulted in continuous community participation for dengue prevention and control. The first was an educational game or "rally" involving children at an elementary school. The 2nd involved a person in a costume who promoted the prevention message with a loud speaker and musical songs designed to increase popular participation. This intervention also had newly developed educational posters. The 3rd intervention was a combination of the 2 previously described strategies. The 4th intervention was the traditional campaign, used for over 10 years, with a loud speaker and distribution of leaflets. The leaflets were distributed in all neighborhoods.

At the same time, polls studied change in the people's knowledge and practice about this subject. When comparing the results of the traditional campaign with the other 3 interventions, there was a significant difference in the reduction of "disposable" breeding sites, depending on which strategy was applied. Results from these interventions suggest that the evaluation of containers (breeding sites), as well as the methods of promotion and education, can help in the identification and elimination of target containers in a cleanup campaign for *Ae. aegypti* control.

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