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

GARY G. CLARK[2] AND MARCO F. SUÁREZ (ORGANIZERS)

San Juan Laboratories, Centers for Disease Control and Prevention
2 Calle Casia, San Juan, PR 00921-3200

ABSTRACT. The third Spanish language symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 59th Annual Meeting in Fort Myers, FL, in April 1993. The principal objective, as for the symposia held in 1991 and 1992, was to increase and stimulate greater participation in the AMCA by vector control specialists and public health workers from Latin America. This publication includes summaries of 25 presentations that were given in Spanish by participants from 8 countries in Latin America, Puerto Rico, and the USA. The symposium included the following topics: ecological, genetic, and control studies of anopheline vectors of malaria; laboratory evaluation and production of biological control agents for *Aedes aegypti*; community participation in the prevention of dengue; and studies of other medically important insects (e.g., *Simulium* and *Triatoma*).

The American Mosquito Control Association (AMCA) is the leading organization of its kind in the world. The AMCA promotes research, needed to understand mosquitoes and other vectors and for control of these arthropods by professionals. In 1993, a Spanish language symposium was held at the 59th Annual Meeting in Ft. Myers, FL. This session was held because of the enthusiastic participation of vector research and control workers from government agencies and academic institutions in Latin America, the financial support of commercial sponsors, and the success of similar sessions held in New Orleans, LA, in 1991 and in Corpus Christi, TX, in 1992. As in prior years, general objectives were to encourage colleagues from Latin America to attend the AMCA meeting, present results of recent studies and projects, promote greater interaction with AMCA members, and stimulate future collaboration in the resolution of vector-borne disease problems in the Americas.

Because of the popularity of this session, it was not possible to include all of the presentations that were submitted to the organizers of this session. As it was, an entire afternoon was devoted to 25 high-quality presentations that were made in Spanish. Simultaneous translation was provided for persons who attended the program but did not understand Spanish. The enthusiasm of the speakers, spirited discussion, and attendance ensured that the symposium once again achieved its objectives.

The support of AMCA members for this unique and innovative session at the Annual Meeting continues to be very good. Special recognition for generous financial support for the 1993 symposium goes to the following sponsors and individuals: Vectec, Inc. (Isaac S. Dyals); the Florida Mosquito Control Association (T. Wainwright Miller, Jr.); ZENECA Public Health (Dr. Julian C. Entwistle); Clarke Mosquito Control Products (John L. Clarke, Jr.); Zoecon, Inc. (Miguel E. Escobar); Summit Chemical Company (Lawrence E. Kase); Beecomist Systems, Inc. (Ed Kutzner); and Sumitomo Chemical America (S. Ohtsuki). This support provided for registration fees, partial travel grants to some participants, and simultaneous translation. Mr. Dennis Moore provided superb assistance in arranging for the translation service and the registration committee was very helpful to international participants.

Funds for the publication of this symposium were provided by the Rockefeller Foundation (Dr. Scott B. Halstead) and ZENECA Public Health (Dr. Julian C. Entwistle). As a result of the positive response from participants and the AMCA leadership, this unique forum will be included in future AMCA meetings.

SYMPOSIUM ABSTRACTS

A study of human factors considered relevant to the transmission and control of malaria in two villages in Honduras (Estudio de los factores humanos considerados relevantes en la transmisión y el control de malaria en dos pueblos de Honduras)

J. C. Stivers and L. A. Rivera

University of South Carolina, School of Public Health, McClellanville, SC, USA and Ministerio de Salud de Honduras, Tegucigalpa, Honduras

A study was performed in Guapinol and Sinai, Honduras, in 1986 to determine which human

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[2] Reprints may be requested from Gary G. Clark, Dengue Branch, CDC, 2 Calle Casia, San Juan, PR 00921-3200.

factors might be relevant to the transmission and control of malaria. The study consisted of the administration of a questionnaire to village residents, observations of the living environment of the residents, and a blood survey to determine malaria prevalence.

In Guapinol, 389 persons living in 74 houses participated in the blood survey, which identified 31 individuals in 21 houses positive for malaria. In Sinai, 436 persons residing in 72 houses were sampled, with 8 people in 7 houses being identified as positive for malaria. Of the 406 individuals surveyed in Guapinol, 46 residing in 28 houses reported a laboratory-confirmed case of malaria during the preceding 3 years. In Sinai, these figures were 437 individuals surveyed with 46 previous cases in 27 houses.

The information gathered during the survey was analyzed using chi-square to determine if there was an association between current or past malaria infections and human habits. The following factors were found to be associated with either current or past malaria infections at $P \leq 0.05$. In Guapinol, current infections were associated with the number of inhabitants per house, with individuals in houses with more than 5 residents being at increased risk. In Sinai, current infections were found to be associated with an individual's sex, wakeup time, and a previous history of malaria infection. Males, individuals with a wakeup time before 0400 h, and individuals with a previous infection were found to be at increased risk of malaria infection. In Guapinol, age group, evening habits or location, occupation, length of residence in the village, and number of inhabitants per house were associated with a history of malaria. Individuals in the age group of 5–14 years, people with mixed indoor and outdoor evening activities, students, people with less than 2 years of residence in the village, and individuals living in houses with more than 5 inhabitants were at a higher risk of having a history of malaria. A history of malaria was associated with evening habits or location, length of residence in the village, and number of inhabitants per house in Sinai also. The specific groups at risk were the same as those in Guapinol.

Observations on the gonotrophic cycle of anophelines (Diptera: Culicidae) in Honduras (Observaciones sobre el ciclo gonotrófico de anófeles de Honduras)

L. Rivera, D. Canales and M. Nelson

División Enfermedades Transmitidas por Vectores, Ministerio de Salud, Tegucigalpa, Honduras and Organización Panamericana de la Salud, Panamá

We studied *Anopheles darlingi* at the Fernando Azcona estate, La Ceiba, Atlandida, Honduras (15°44′24″N, 86°51′36″W). Mosquitoes were captured with human bait and marked with "Dayglo" fluorescent powder. After release, attempted recapture of marked mosquitoes used human bait. In the laboratory, species were identified, abdominal appearance was recorded, and ovaries were dissected to determine the physiological age and follicular development. These activities were carried out between 1830 and 2330 h bimonthly for 6 consecutive days from May 26, 1989, to May 23, 1990. Of 11,332 *Anopheles albimanus* and 10,187 *An. darlingi* marked, 1.1 and 1.3% of these species, respectively, were recaptured. A significant correlation was found between the abdominal appearance and follicular development for both *An. albimanus* and *An. darlingi* ($\chi^2 = 3.58$, $P < 0.05$, df = 1; $\chi^2 = 4.00$, $P < 0.025$, df = 1, respectively). In both species, we found that the nullipars had bloodfed 2 or more times within the gonotrophic cycle. For both species, the duration of phase 2 of the gonotrophic cycle was 60.4 ± 20.4 h, with a maximum for the first cycle of 120 h. The second gonotrophic cycle began with the appearance of Christophers' second stage.

Population dynamics of *Anopheles* sp. from malaria endemic areas of coastal Colombia (Dinámica de población de *Anopheles* sp. de zonas costeras endémica de malaria en Colombia)

R. Valderrama, S. Franco, S. Blair, M. F. Suárez, L. Osorio, M. C. Correa and O. Gallo

Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Antioquia y Servicio de Erradicación de la Malaria, Medellín, Colombia

We analyzed ecological aspects that affect the distribution and population dynamics of the most important vectors in a malaria-endemic area from coastal Colombia. The objectives were to identify local species and their distribution and densities in macro- and micro-ecological habitats, and determine how several ecological variables affected their population dynamics.

The area was categorized according to land-use practices, type of soil, elevation, air temperature, relative humidity, type of hut (percentage of wall protection that prevents the entry of mosquitoes), and number of inhabitants per dwelling. Anophelines were collected between 1800 and 2400 h with indoor and outdoor human baits and from resting sites inside the hut. The number of anophelines per dwelling per day (ADD) and

the number of anopheline per dwelling per year (ADY) were calculated.

During one year of study, 3,356 adult anophelines were collected from 44 dwellings near the Uraba Gulf on the Atlantic coast. *Anopheles albimanus* and *Anopheles nuneztovari* represented 70% of the collections and 19% were *Anopheles punctimacula, Anopheles pseudopunctipennis,* and *Anopheles darlingi,* all important malaria vectors. The remaining 11% were other anophelines with minor or no epidemiological importance as malaria vectors. The distribution of anophelines and their population dynamics were different in the 3 ecological subzones (littoral, hills, and intermountains). The ADD in the littoral areas was greater than in the intermountain subzone. The largest ADY was found in the intermountain subzone (ADY = 119), whereas the ADY in the littoral was 74 and in the hills was 15. The ADD was similar to the ADY in these subzones. The percentage of unprotected dwellings was above 40 but below 60 in subzones with higher ADD. The average number of inhabitants per dwelling was 5. The outdoor collections of anophelines yielded 85% of the total. These findings reflect a high risk for the transmission of malaria in this area.

Feeding index of anophelines in western Venezuela (Indice de alimentación de anofelinos en el occidente de Venezuela)

Y. Rubio-Palis, C. F. Curtis and R. A. Wirtz

División de Investigaciones, Escuela de Malariología y Saneamiento Ambiental, Venezuela; Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK; and Department of Entomology, Walter Reed Army Institute of Research, Washington, DC, USA

The tendency to feed on human blood is a useful indicator in determining the relative importance of *Anopheles* species as vectors of malaria. These feeding patterns could also be useful in the epidemiological assessment of control activities as a comparative measure of the effect of residual insecticide applications upon the degree of contact between vector and man.

To assess the feeding patterns of anophelines in a vivax malaria endemic area in western Venezuela, mosquitoes resting on vegetation in 3 villages were collected with an aspirator between 0600 and 0800 h, 4 days per month at each village for 14 months. After species identification, anophelines were kept dry over silica gel until assayed by ELISA. The human and domestic animal populations in the villages were censused.

The feeding index (FI) (i.e., the observed proportion of feeds on one host with respect to another divided by the expected comparative proportion of feeds on the 2 hosts [Kay et al. 1979]) was calculated for the 4 most common species: *Anopheles nuneztovari, Anopheles triannulatus, Anopheles oswaldoi,* and *Anopheles albitarsis,* based on the results of the ELISAs and questionnaires.

The FI was found to be different for each species in each village. In Guaquitas, where there were 487 bovines and 44 people, the FI for all 4 species was higher than 1.0 (i.e., there was apparently preferential feeding on humans). These results contrast to those in Jabillos where there were 282 bovines and 303 people and where *An. nuneztovari, An. albitarsis,* and *An. triannulatus* apparently fed preferentially on bovines. In Caño Lindo (320 bovines and 114 people), *An. nuneztovari* and *An. albitarsis* fed preferentially on humans. The contrasting results obtained in these villages may be explained by the larger number of cows belonging to the inhabitants of Guaquitas and Caño Lindo, kept closer to where the mosquitoes were collected than at Jabillos.

Predictive analysis of *Anopheles triannulatus* abundance in western Venezuela (Análisis predictivo de la abundancia de *Anopheles triannulatus* en el occidente de Venezuela)

M. Oviedo and J. V. Scorza

Centro Trujillano de Investigaciones Parasitológicas "J. W. Torrealba," Trujillo, Venezuela

For 2 consecutive years, the abundance and indoor and outdoor human biting activity of *Anopheles triannulatus* was studied monthly between 1990 and 2200 h in western Venezuela (9°34'N, 70°37'W). The population of *An. triannulatus* increased with a rainfall of 100 mm during the first half of the year and peridomiciliary biting activity peaked between 1900 and 2000 h.

Measurements of temperature, relative humidity, and pluvial rainfall were compared with local meteorological data obtained from a station located 2 km from the study site. Regression analysis and multiple stepwise regression were used to compare the biting rates of *An. triannulatus* with the accumulated rainfall; discriminating between strong (PF) and weak rains (PD), taking into account if they were above or below the annual average; the rainfall on the day of capture (PC); if rainfall occurred the day before

the capture (PA); the relative humidity (HR); and temperature (T°C).

Multiple regression analysis showed the possibility of predicting with a 76% reliability ($P = 0.05$) the number of mosquitoes that bite according to the following regression equation: number of mosquitoes = 243.6 + 0.52619PC − 2.644T°C − 4.4373PD − 5.8526PF − 1.2608HR − 4.6661PA + 0.2023PP. The multiple stepwise regression suggested that under weak precipitation conditions, with a reliability of 88% ($P = 0.05$), the number of mosquitoes that will bite follows the following equation: number of mosquitoes = −81.5 + 5.244PD. This last equation needs to be confirmed under field conditions.

A study of the genetic structure of a population of *Anopheles nuneztovari* (Estudio sobre la estructura genética de *Anopheles nuneztovari*)

Y. Rangel and L. E. Munstermann

Universidad Central de Venezuela, Caracas, Venezuela and Yale University School of Medicine, New Haven, CT, USA

Anopheles nuneztovari is the main malaria vector in western Venezuela and northern Colombia. Some authors have suggested that *An. nuneztovari* is a sibling species complex, based on cytogenetic studies, biting activity, and medical importance. The objective of this study was to expand earlier isozyme analyses of *An. nuneztovari* by examining 18 loci from 5 populations in its geographical range. Three of these populations were from La Lengueta de Barinas (western Venezuela, southern Cordillera de los Andes). One was from La Fría (northern Cordillera de los Andes) and the last was from Belem, Pará State, Brazil.

The 3 populations from La Lengueta de Barinas showed little genetic differentiation, as indicated by the Fisher's F-statistic (F[ST]) of 0.023. Only the electromorphs of hydroxyacid dehydrogenase (HAD) differed significantly from a Hardy-Weinberg equilibrium. The La Fría population had striking differences at the malate dehydrogenase (MDH) locus, although the overall F(ST) of 0.066, with respect to the La Lengueta populations, was low. The sample from Brazil was genetically distinct, including a diagnostic allele at the glycerol dehydrogenase (GCD) locus and high frequency differences with MDH and HAD isozymes. These were reflected by an F(ST) of 0.432. The high degree of differentiation across the range of *An. nuneztovari* indicates that it is a complex of cryptic species.

Larval ecology of the malaria vector, *Anopheles pseudopunctipennis*, in the foothills area of Tapachula, southern Mexico (Ecología de las larvas del vector de malaria, *Anopheles pseudopunctipennis*, en el area de las colinas de Tapachula al sur de México)

I. Fernández-Salas, D. R. Roberts, M. H. Rodríguez and C. F. Marina-Fernández

Universidad Autónoma de Nuevo León, San Nicolás de los Garza, México; Department of Preventive Medicine and Biometrics, Uniformed Services University of Health Sciences, Bethesda, MD, USA; and Centro de Investigaciónes de Paludismo, Tapachula, México

A study of the population dynamics of *Anopheles pseudopunctipennis* larvae was conducted in the foothills near Tapachula, Mexico. Systematic surveillance of wet season and dry season habitats was conducted in 1990 and 1991. Samples were taken along a transect of the Coatan River to quantify habitat availability and population densities of larvae during the dry season from December to May. During the wet season (July–November), larvae were most abundant in temporary habitats, such as seepage springs (25%), rain pools (15%), and pools in streams (15%) and river margins (15%). These transitory habitats produced more than two-thirds (70%) of the larvae collected during the wet season. The temporary habitats disappeared during the dry season, concurrent with increasing densities of larvae in habitats within the transect. The great abundance of the dry season riverine habitats, *viz.*, small pools with filamentous algae, resulted in peak densities of host-seeking adult populations in villages along the river. During both seasons, there were significant associations in the presence and abundance of larvae with habitats containing filamentous algae, and secondarily with aquatic and semiaquatic plants. There was a significant correlation between mean number of larvae per habitat type and mean number of breeding sites in the transect. Overall, populations of *An. pseudopunctipennis* larvae were very abundant during the dry season and relatively uncommon during the wet season.

Pilot production of *Bacillus thuringiensis* var. *israelensis* using whole coconuts and field applications for biological control of *Anopheles* in malaria endemic areas of Peru (Producción piloto usando cocos completos y aplicación en el campo *Bacillus thuringiensis* var. *israelensis* para el control biológico de *Anopheles* en áreas endémicas de malaria en el Perú)

P. Ventosilla, C. Ruiz de Somocurcio, H. Guerra, D. Marín and I. Columbus

Instituto de Medicina Tropical "Alexander von Humboldt," Universidad Peruana Cayetano Heredia, Lima, Perú

About 56% of the world, 39% of the Latin American, and 37% of the Peruvian population are at risk to malarial infections. In Peru, malaria is distributed in several endemic areas along the coast and in the jungle. Despite increased vector resistance and ecological damage, insecticides are still the key tools for malaria control. Consequently, the use of specific entomopathogens for the control of insect vectors of disease is becoming increasingly attractive. *Bacillus thuringiensis* var. *israelensis* H-14 (*B.t.i.*), one of the better known agents, acts through a cytolytic protein produced during sporulation, which destroys the larval midgut epithelium.

We are developing a simple procedure to be used by rural communities for local production and application of *B.t.i.* using resources present in malaria endemic areas. Laboratory studies have demonstrated that artificial media and coconut water, either in glass flasks or within the fruit, produced *B.t.i.* at the same rate and yielded the same total quantity. Toxic activity against both 3rd and 4th instar *Culex* and *Anopheles* larvae was similar. Field trials showed the presence of viable *B.t.i.* spores in seeded natural ponds in endemic areas for 21 days (Lima) and 18 and 25 days in warmer areas (Piura, Yurimaguas).

The ability of local communities in endemic areas to control malaria through production and use of *B.t.i.* appears to be feasible.

Field evaluation of pirimiphos methyl and lambda-cyhalothrin for *Anopheles aquasalis* control in Sucre State, Venezuela (Evaluación de campo de pirimifos metil y lambda-cihalotrina en el control de *Anopheles aquasalis* en el estado de Sucre, Venezuela)

J. Berti and J. L. Pérez

Dirección de Endemias Rurales, Ministerio de Sanidad y Asistencia Social, Maracay, Venezuela

Malaria in Sucre State, Venezuela, is transmitted predominantly by *Anopheles aquasalis*, and has increased steadily from 4,998 cases in 1988 to 8,231 cases in 1991. A trial was conducted from September 1991 to February 1992 in 4 Sucre villages with a total population of 3,662 residents to evaluate 3 insecticides that had not been used operationally for malaria control in Venezuela. In Barcelo, a single intradomiciliary spray of lambda-cyhalothrin 10 WP was applied to 30 mg AI/m^2. In Cachipal, a similar application of pirimiphos-methyl 50 EC was made at 2 g AI/m^2. In Quebrada de la Niña, ULV space-sprays of lambda-cyhalothrin 2.5 EC were applied weekly at 1 g AI/ha and in El Paujil, both an intradomiciliary application of pirimiphos-methyl 50 EC at 2 g AI/m^2 and weekly space-sprays of lambda-cyhalothrin 2.5 EC at 1 g AI/ha were carried out. World Health Organization cone bioassays and susceptibility tests were conducted. Human bait counts were also made on a weekly basis, and mosquitoes were examined for parity status. The number of malaria cases reported was reduced in all 4 villages in comparison with the previous year. The reduction was greater in the 2 areas that received space applications, and the most rapid decline of all was observed in El Paujil, which had received both treatment regimes. All the products tested were effective in reducing malaria, especially the space-spray applications of lambda-cyhalothrin 2.5 EC.

Statistical evaluation of the man-biting activity of *Simulium ochraceum* to estimate *Onchocerca volvulus* transmission in a hyperendemic focus from southern Mexico (Evaluación estadística de la actividad de picadura de *Simulium ochraceum* para estimar la transmisión de *Onchocerca volvulus*, en un foco hiperendémico del sur de México)

M. A. Rodríguez-Pérez and F. Reyes-Villanueva

Centro de Investigaciones Ecológicas del Sureste, San Cristóbal de las Cases, Chiapas and Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, México

The human-bait landing collection is the method traditionally used to monitor the seasonal abundance of vector simuliids. Its use has been empirical, however, because there is no statistical evaluation with a reliability level of the collection time and baits (i.e., sample size required), to estimate a mean number of *Simulium ochraceum* females infected with *Onchocerca volvulus*. In the onchocerciasis control programs of Guatemala and southern Mexico, entomologists used a sample size (n) depending on availability of resources (time and money). They worked with a conjectural n because the estimates of *S. ochraceum* populations and *O. volvulus* transmission levels lacked a probabilistic reliability level.

This study was to evaluate the applicability of the presence–absence method to calculate the

sample size required in collection days and human baits, and to estimate the mean number of *S. ochraceum* females infected with *O. volvulus* larvae.

The study was conducted at Las Golondrinas village, Acacoyagua (14.8°N, 92.3°W) at 800 m elevation in the Sierra Madre of Chiapas. Monthly collections were made from September 1990 to September 1991. Each sampling included 8 days of collections using 2 human baits (community volunteers): one located in the center of the village and the other in a coffee plantation located 200 m from the village. Collections were made from 0800 to 1800 h, and only the black flies that landed during the first 30 min of each hour were caught. All *S. ochraceum* specimens were dissected immediately and parity and *O. volvulus* larval stages were determined according to the method of Porter and Collins (1984).

The sampling model of Gerrard and Chiang (1970), known as the "presence–absence" method, was applied to the data. This model is independent of the spatial distribution pattern, and describes the relationship between the mean number of *S. ochraceum* females infected with *O. volvulus* larvae by collection period (5-h interval and 24-h period), and the proportion (p) of collection periods positive for infected females. In this relationship, the number of days and collection periods, as components of the sample size, can be used to express the collection effort required to estimate the mean number of the *O. volvulus*-infected females, or the infective females (with the L3 stage), at any statistical reliability level.

Forty-three days of collections with 2 baits yielded a daily mean of 0.13 infective females (mean SE = 0.40). This transmission level was equivalent to $P = 0.13$ in a preliminary sampling of 8 days, if there was one infective female (1/8). This sample size can be used in an onchocerciasis control program with ivermectin, to assess the transmission level before and after treatment. We suggest that 3 collections of *S. ochraceum* be made, distributed in the 3 months of the year when *O. volvulus* transmission is highest in the community.

Distribution of triatomids in two villages from Nuevo Leon, Mexico (Distribución de triatomineos en dos pueblos de Nuevo León, México)

L. C. Galaviz-Silva, C. Lara-Campos, M. Ramos-Guerra and F. Segovia-Salinas

Laboratorio de Parasitologia, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo Leon, San Nicolás de los Garza, México

Triatomine surveys were conducted in Mexico in 1985–89 and in 1991. During the first survey, 66 *Triatoma gerstaeckeri* (35 adults and 31 nymphs) and 14 *T. lecticularia* (6 adults and 8 nymphs) were found. This infestation occurred in 36 of the 46 households surveyed in the villages of Vaquerias, Huizachito, La Gloria, and Delicias. Entomological indicators reflected an infestation index, II = 48; density index, DI = 143; crowding index, HI = 300; dispersion index, DDI = 100; colonization index, CI = 50; and natural infection index, NII = 36. The highest NII for *T. gerstaeckeri* was in Delicias (100) and for *T. lecticularia* in La Gloria (50). These 2 species are included in the list of triatomines that serve as household vectors of Chagas' disease in Mexico. Our results support their role as potential vectors of Chagas' disease and the need to include assessment of entomological indicators for better understanding of the epidemiology of American trypanosomiasis.

The purpose of the second survey was to determine the species of triatomids present in 10 villages of General Teran, Nuevo Leon, Mexico; their entomological indices; seasonal distribution; sex ratio; and degree of association with human dwellings. The 2 species captured were *T. gerstaeckeri* (192) and *T. lecticularia* (9). *Triatoma gerstaeckeri* was found in 10 villages (DDI = 80) and 15 households (II = 24); *T. lecticularia* was found in only 4 villages (DDI = 40) and 5 households (II = 5). The *Trypanosoma cruzi* infection rates were similar in both species (NII = 28 and 33, respectively). The greatest number of individuals were trapped during the month of June (90 *T. gerstaeckeri* and 2 *T. lecticularia*). Of 192 *T. gerstaeckeri* collected, 124 (65%) were females and 68 (35%) were males; only 8 female (88%) and 1 male (11%) *T. lecticularia* were captured. Both species were considered as peridomiciliary triatomids, adapted to human dwellings. Of these, 181 (97%) of the *T. gerstaeckeri* and 6 (67%) of *T. lecticularia* were captured around human dwellings.

Survey of natural parasites of Culicidae of Buenos Aires Province, Argentina (Encuesta de parasitos en *Culicidae* de la provincia de Buenos Aires, Argentina)

J. J. Garcia, R. E. Campos and A. Macia

Centro de Estudios Parasitológicos y de Vectores (CEPAVE), La Plata, Argentina

A survey for parasites of culicids was conducted in the southeastern sector of a subtropical forest in Punta Lara, Buenos Aires Province, Argentina. Mosquito populations were sampled

weekly for immature stages and every 15 days for adults from August 1989 to August 1991.

Larvae and pupae (n = 48,212) of 19 culicid species were examined for parasites. Fourteen culicid species were infected and parasitized by: 4 species of fungi (*Achlya* sp., *Coelomomyces reticulatus, Geotrichum candidum,* and *Smittium morbosum*); microsporidia of the genus *Amblyospora*; and a mermithid nematode, *Strelkovimermis spiculatus.* Infection rates of these parasites over the 2-year study were 26% for *S. spiculatus,* 12% for *S. morbosum,* and less than 1.5% for the other 3 parasites combined. Higher prevalence of parasitism occurred during the spring and fall months when host populations were also high. Infections with *Amblyospora* spp., *C. reticulatus,* and *S. spiculatus* were lethal for host larvae; in contrast, *S. morbosum* caused death in some hosts but not in others. Presence of *G. candidum* was never related to the death of the host larva.

Female adults of 36 species of culicids were collected; however, only the 6 most abundant species (n = 3,357) were examined for parasites. Resting sporangia of *Coelomomyces* sp. (0.4%) and haplosporidian-like spores (0.2%) were found in ovarian tissue of adults. Females infected with *Coelomomyces* sp. showed a massive destruction of the ovarian tissue. Filarid nematode larvae were found in the hemocoel and Malpighian tubules of 2.4% of the adults. Also, *S. spiculatus* was found in the hemocoel of one female and conidia were observed in the hemolymph of 4.4% of the adults. No evidence of tissue damage was observed in ovaries when haplosporidian-like spores or hyphomycete conidia were present. Research is being conducted to evaluate the impact of these parasites as natural biocontrol agents of culicids.

Effect of Bactimos on the predatory capacity of *Buenoa* sp. upon *Culex quinquefasciatus* larvae (Efecto de Bactimos sobre la capacidad depredadora de *Buenoa* sp. sobre larvas de *Culex quinquefasciatus*)

M. Reyna, A. Solis, N. Gorrochotegui and E. A. Rebollar

Laboratorio de Entomologia Medica, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, México

The synergetic effect of Bactimos (*Bacillus thuringiensis* var. *israelensis*) was evaluated on the predatory capacity of *Buenoa* sp. (Hemiptera: Notonectidae) upon *Culex quinquefasciatus.* First and 2nd larval instars were used for the first test and 3rd and 4th instars in the second bioassay. Larvae were placed into 1-liter glass containers filled with 750 ml of dechlorinated water (pH = 6.5) and each predator was exposed to 10 larval densities. In a second experiment, the predator and Bactimos at the recommended dosage (9.3 g/m^2) were added. The number of prey consumed was recorded after a 24 h period, using 5 repetitions per density. Both treatments were conducted at a 14:10 h light : dark photoperiod, with an average temperature of 24°C. Results were analyzed with a linear regression for the first bioassay indicating that $Y = 1.83571 + 0.53204X$, for the 1st and 2nd instars, whereas for the 3rd and 4th larval instars $Y = 2.01544 + 0.42144X$. Using Holling's functional response equation, a searching capacity of (a') = 0.034155, and a handling time of (th) = 0.23999 were determined for 1st and 2nd instars; and a' = 0.02589, th = 0.16460 for 3rd and 4th instars. On the other hand, using Rogers's equation, results were a' = 0.081822, th = 0.44205 for the 1st and 2nd instars, and a' = 0.05348, th = 0.44796 for the 3rd and 4th, respectively. Combined linear regression for the predator and Bactimos indicated $Y = 0.35253 + 0.98374X$ and $Y = 1.8256 + 0.75461X$ for the 1st and 2nd, and the 3rd and 4th instars, respectively; with Holling's equation, a' = 0.03813, th = 0.03546 for 1st and 2nd instars, and a' = 0.04204, th = 0.12224 for 3rd and 4th instars. Similarly for Rogers's equation a' = 0.16313, th = 0.02112 for 1st and 2nd instars, and a' = 0.33180, th = 0.52410 for 3rd and 4th instars. Bactimos alone was also tested on *Cx. quinquefasciatus* larvae to determine the mean percentage of mortality among the treatments. First and 2nd instar mortality for the predator alone was 70.6%, Bactimos and predator 94.5%, and Bactimos alone 99.2%. For 3rd and 4th instars mortality was 57.2, 91.2, and 66.2% for the predator, Bactimos and predator, and Bactimos alone, respectively. Holling's equation results indicated that predatory action increased as the larval density increased. In general, the action of the predator *Buenoa* sp. and Bactimos combined was more effective than the other 2 treatments. We observed that *Cx. quinquefasciatus* larvae were more susceptible to the predatory action of *Buenoa* sp. when Bactimos was present, perhaps because Bactimos reduced larval strength and therefore antipredatory responses were diminished.

Dengue hemorrhagic fever epidemic in Bucaramanga, Colombia, 1992 (Epidemia de dengue hemorrágico en Bucaramanga, Colombia, 1992)

J. E. Luna and G. León

Table 1. Survey and collection results.

Neighborhood	Before clean-up campaign			After clean-up campaign		
	Houses inspected	House index	Adult index[1]	Houses inspected	House index	Adult index[1]
Provenza	40	8	20	93	13	14
Fontana	16	4	25	20	3	15
Asturias I	20	6	30	26	4	15
Diamante II	16	2	12	70	7	10

[1] Percentage of houses inspected with adult *Aedes aegypti.*

Table 2. Survey and collection results.

Neighborhood	Before ULV			After ULV		
	Houses inspected	House index	Adult index[1]	Houses inspected	House index	Adult index[1]
Provenza	28	17	60	28	5	18
Fontana	17	12	70	17	3	18
San Luis	31	15	48	32	10	31
Diamante II	33	4	12	33	4	12

[1] Percentage of houses inspected with adult *Aedes aegypti.*

Instituto de Salud, Bucaramanga, Colombia

On March 25, 1992, physicians at the Instituto de Salud de Bucaramanga reported the first case of dengue hemorrhagic fever (DHF) in Bucaramanga city that met World Health Organization (WHO) criteria. The metropolitan area of the city has 800,000 inhabitants, distributed in 14 "comunas," each with a different number of neighborhoods. The first DHF case was reported from comuna 10. From March 25 until late November, 412 DHF cases were reported from the metropolitan area. In May and June, 163 and 140 DHF cases were recorded, respectively. During the outbreak the National Institute of Health in Bogotá identified dengue 2 as the infecting serotype.

Immediately after the first DHF case was reported, a larval survey was conducted in the 14 comunas of the city. The house index ranged from 2% in comuna 12, to 20% in comuna 10, to 33% in comuna 1. After that survey, a clean-up campaign was conducted and an ultra-low volume (ULV) application of 97% Malathion® was made and evaluated in the neighborhoods of comuna 10. The evaluation included larval surveys and landing collection with human bait. The results are summarized in Tables 1 and 2.

The ULV application and the clean-up campaign were carried out by health authorities from the central government level without community participation. The results showed that control with poor community participation only produced a temporary reduction in *Aedes aegypti* infestations. After the DHF outbreak, an action project with community participation was initiated. The objective was to develop an active educational program and get greater community participation. We prepared 3 videos: one with puppets for children; another with information for the general public; and an educational video for orientation of the 500 high school students who visit houses, conduct surveys, and disseminate educational messages about dengue prevention each year. This project received support from a grant from the Rotary Foundation through the Rotary Club from San Juan, PR, to the Bucaramanga-Chicamocha Club and the Instituto de Salud.

The impact of substandard piped water supply on *Aedes aegypti* density in Venezuela (Deficiencia de agua de acueducto y densidad de *Aedes aegypti* en Venezuela)

R. Barrera, J. C. Navarro, J. D. Mora, D. Domínguez and J. González

Instituto de Zoología Tropical, Universidad Central de Venezuela, Caracas and Ministerio de Sanidad y Asistencia Social, Maracay, Venezuela

Previous research showed a link between *Aedes aegypti* infestation indices and a deficiency in piped-water supplies in 2 coastal towns in Venezuela. Here, we extend the analysis to include 30 towns along the Venezuelan coast, where

we sampled 3,000 houses and identified all containers with and without *Ae. aegypti*. The hypothesis was that a deficient piped water supply was positively associated with the infestation indices. Along with the entomological survey, we asked the householders the following questions regarding their piped-water supply: source of water (pipes inside house, pipes only outside house, none), frequency of interruptions in water service (never, seldom, bi-weekly, weekly, daily), and the length of the interruptions (hours, days, weeks, months).

The overall house index was 55% (range: 24–85%), the mean Breteau index was 118 (range: 38–263), and the mean container index was 24% (range: 11–39%). The order of breeding site positivity was: 208-liter metal drums (40%), house plants (37%), discarded tires (35%), concrete water tanks (21%), cans (14%), miscellaneous containers (12%), and animal drinking pans (8%). Kruskal-Wallis analyses revealed significant differences ($P < 0.001$) among medians of the number of total positive containers ($\chi^2 = 36.7$; $n = 2,996$) and positive water-storage containers ($\chi^2 = 67.0$; $n = 2,996$) with regard to the frequency of interruptions. Houses with daily and weekly interruptions had greater numbers of positive breeding places. The statistical analyses also showed significant differences ($P < 0.001$) among the medians of total breeding places ($\chi^2 = 42.8$; $n = 2,760$) and water storage breeding places ($\chi^2 = 114.3$; $n = 2,760$) per house, across the duration of water supply interruptions. The number of breeding places per house increased with the length of interruptions. Most of the water-storage containers (79%), and most of the positive water-storage containers were metal drums (88%). The data showed that most houses had piped water at some time, so that the main problem with the service of piped water seems to be its unreliability (frequent and lengthy interruptions), which caused residents to store water and stimulate *Ae. aegypti* production. We acknowledge financial support for this study from PAHO and CONICIT.

Advances in the *Aedes aegypti* community-based control project in El Progreso, Honduras (Advances en el proyecto de control de *Aedes aegypti* con base comunitaria en El Progreso, Honduras)

E. Fernandez, I. Lagos and C. Sherman

Proyecto Control Integrado de Dengue, Ministerio de Salud de Honduras, El Progreso, Honduras

In June 1991, the Integrated Community-based *Aedes aegypti* Control Project in El Progreso, Honduras, began gathering basic information about the city and its social and entomological characteristics regarding *Aedes aegypti*. The city of Santa Rita about 25 km away was surveyed and used as an untreated area.

The main objective of this project was to test the efficacy of participatory methods with the community and to stimulate and mobilize the population to eliminate/control *Ae. aegypti* breeding sites, reduce entomological indices (i.e., the house index), and produce a sustainable effort in the reduction of factors that enhance *Ae. aegypti* infestations (i.e., inefficient refuse disposal or trash accumulations on patios).

Ten neighborhoods (with house indices from 12 to 51) were included in the community promotion program. The neighborhoods were mapped, divided in zones, and taken through a 4-phase promotion plan to stimulate the formation and continuation of volunteers in Integrated Health Groups (GSI).

During the first 6 months of 1992, the GSI activity resulted in a 32–92% decrease in house indices, compared with Santa Rita (untreated area), a significant difference ($P < 0.05$). Besides the reduction in house indices, the GSI have developed other activities in these neighborhoods, such as clean-up campaigns, a weekly refuse collection system, educational material distribution, and patio inspections among others, as part of their program.

Community participation program for dengue control in the Magdalena River valley, Antioquia, Colombia (Participación comunitaria para el control del dengue en 3 municipios del Valle Medio del Magdalena, Antioquia, Colombia)

G. Alvarez, Y. López, Y. Montiel, A. Bolívar, J. Velez and J. P. Escobar

Servicio Seccional de Salud de Antioquia, Medellín, Colombia

During 1992, 1,329 dengue cases were reported in the Department of Antioquia. Of these, 441 (33%) were registered from Puerto Berrio, Yondo, and Puerto Nare, 3 municipalities in the Magdalena River valley where the population is about 28,000. Dengue 2 virus was isolated by our laboratory from this area. At the same time, 17 cases of dengue hemorrhagic fever (DHF) were confirmed in the Department of Antioquia; 2 were from Puerto Berrio. The 55-gallon metal drums usually used for water storage were the most numerous breeding sites for *Aedes aegypti* mosquitoes. During the outbreak of DHF, dengue surveillance was increased, a health education program emphasizing source reduction was

implemented, periodic "clean-up" campaigns and recycling activities were conducted, and ULV Malathion® was applied. The objective of this study was to develop and initiate an action plan for dengue control with community participation. To involve the community in the control strategies, residents were invited to assist in several workshops where they learned to minimize their contact with *Aedes aegypti*. The community cooperated in different activities and an action group helped design educational materials. Entomological surveys were used to evaluate this program. We expect that these strategies will break dengue transmission in the area. If so, we will review and evaluate these interventions for use in other areas. This project has been supported by a Rotary International Grant through the Rotary Clubs from San Juan, PR, and Medellin, Colombia, and the Servicio Seccional de Salud de Antioquia.

Aedes aegypti control without chemicals in cemeteries in Puerto Rico (Control de *Aedes aegypti* en cementerios en Puerto Rico sin usar insecticidas)

F. Medina, M. F. Suárez and G. G. Clark

Puerto Rico Department of Health, Caguas; Center for International Community-based Health Research, Johns Hopkins University, Baltimore, MD, USA; and Dengue Branch, NCID, CDC, San Juan, PR

Cemeteries are important foci of *Aedes aegypti* production and often have many larval breeding sites. The traditional strategy for control in these areas in Puerto Rico has been the application of temephos in the vases by local environmental health inspectors. During recent years, the budget for mosquito control programs has been reduced and has diminished the availability of insecticides and operational personnel.

Twenty-six cemeteries in 7 municipalities in Caguas, PR, were monitored from June 1988 to July 1992. Each cemetery was visited every 4 months by 2 inspectors. During the visit, the importance of various containers in producing *Ae. aegypti* was emphasized with the cemetery administrator, including the elimination of water for fresh-cut flowers. All vases and flower pots were inspected and each container with water was filled with sand. During the last 2 years, the number of containers that needed to have sand reapplied was minimal and the level of enthusiasm for this control strategy by cemetery authorities was high. Some cemeteries now have a sign at the entrance explaining how to prevent dengue and promoting the use of sand. Through July 1992, 25 cemeteries were completely negative for *Ae. aegypti* larvae. Mosquito surveillance associated with educational messages and application of sand to the containers is an excellent strategy for *Ae. aegypti* control with minimal personnel and without use of insecticides.

The turtle, *Pseudemys scripta*, as a biological control agent for *Aedes aegypti* in El Progreso, Honduras (La tortuga, *Pseudemys scripta*, como agente de control biológico de *Aedes aegypti* en El Progreso, Honduras)

C. Sherman, E. Fernández and H. Portillo

Proyecto Control Integrado del Dengue, División de Enfermedades Transmitidas por Vectores, Ministerio de Salud, El Progreso, Honduras

This study was part of a project conducted by the Honduran Ministry of Public Health on the effectiveness of biological control methods for *Aedes aegypti*, the principal vector of dengue fever. Following previous observations of turtle (*Pseudemys scripta*) predation on *Ae. aegypti* larvae, field trials were begun in March 1992 to study turtle survival, predatory effect on mosquito larvae, social acceptability, and turtle presence in treated containers through September 1992. Turtles were introduced into cement cisterns ("pilas") used to store water for household purposes and laundry and into 55-gallon metal drums used for storage of nondrinking water in 3 communities. The predatory activities of turtles and procedures for basic care of turtles in these containers were explained to the housewives.

The average survival time of turtles in these containers during the 7-month observation period was 10–63 days. Of the 79 turtles introduced, 73% disappeared; the majority were lost because the household members had insufficient information about their management. Only 23% were still present at the end of the observation period and 4% had died. With the high degree of acceptability of the turtles by residents, there is a need to achieve more participation on the part of the community in the distribution and maintenance of turtles in these containers.

Laboratory trials of prey selectivity of *Macrocyclops albidus* and *Mesocyclops longisetus* (Copepoda: Cyclopoidae) (Selectividad de presas de *Macrocyclops albidus* y *Mesocyclops longisetus* en ensayos de laboratorio)

S. M. Pérez-Serna, N. Ornelas-Navas, H. Quiróz-Martínez, M. H. Badii and V. Vásquez-Carreón

Facultad de Ciencias Biológicas,
Universidad Autónoma de Nuevo León,
San Nicolás de los Garza, México

Although the searching capacities of *Macrocyclops albidus* and *Mesocyclops longisetus* as mosquito predators have been evaluated, their preferences in complex prey systems have not been analyzed. The objective of this research was to evaluate the selectivity or preference of 2 copepod species in systems with 2 prey species to obtain important information about their potential as biocontrol agents.

Individual females of *M. albidus* and *M. longisetus* were exposed to 1, 2, 4, 8, 16, and 32 1st instar *Aedes aegypti* or *Culex pipiens* mosquito larvae. The tests were performed in small glass containers with 750 ml of nonchlorinated water, with 5 replicates. After 24 h of exposure, the number of prey still alive was recorded.

Results were analyzed by Functional Response models of Holling (1959), Romaya (1971), and Rogers (1972); Ivlev's Index of Electivity (1961); and Jacob's Index of Selectivity (1974). The best "selectivity" value was the higher value of searching capacity and lowest handling time between copepod species.

According to Functional Response models, *M. albidus* showed preference for *Ae. aegypti,* but selectivity and electivity indices were near 0 (no clear preference) and for *Cx. pipiens* larvae the indices were negative (no preference). The second species studied, *M. longisetus,* had no clear preference in functional response models, although it favored *Cx. pipiens,* whereas electivity and selectivity indices were higher toward *Ae. aegypti.*

Life tables of *Mesocyclops longisetus* (Copepoda: Cyclopoidae) in the laboratory (Tablas de vida de *Mesocyclops longisetus* bajo condiciones de laboratorio)

B. Gonzalez-Rodriguez, M. H. Badii,
H. Quiróz-Martínez and
G. A. Rodríguez-Almaraz

Facultad de Ciencias Biológicas,
Universidad Autónoma de Nuevo León,
San Nicolás de los Garza, México

Life tables provide a graphic representation of population dynamics (survivorship and reproduction of an organism) and the optimal time to manage a population with biological control programs. In the last 2 years, we have tested copepods to control *Aedes aegypti. Mesocyclops longisetus* has been evaluated in laboratory and under field conditions but quantitative information about its biology and reproduction is rare.

Life tables with nauplii of the same cohort were prepared. Nauplii were introduced individually in assay tubs with *Paramecium caudatum* as food, a 12 h:12 h light : dark regime, and regulated temperature, and mortality was recorded every 24 h. When copepods reached the adult stage, 2 pairs were separated and their progeny were counted daily until the last female died. The results were analyzed by life tables, survivorship tables, and fecundity tables.

The survivorship value (l_x) was higher in adults between 10 and 27 days of age and the fecundity value (m_x) was higher in copepods 18 and 55 days of age, with 3–4.7 nauplii per female. The total progeny per female per day ($l_x \cdot m_x$) was higher in 18-day old females (2.1 nauplii). The life expectancy (e_x) was 34.1 days and the percentage of mortality (q_x) was greatest in the early stages of development. The stable age distribution (C_x) was 17% in time zero at the beginning of the test. The net reproductive rate (R_o) was 21.3 nauplii per generation and the finite rate of increase (λ) was 1.2 nauplii per day. The intrinsic rate of increase (r_m) was 0.15 nauplii.

Life expectancy and habitat quality requirements for mass production and storage of *Mesocyclops longisetus* (Expectativa de vida y calidad del habitat requerido para la producción masiva de *Mesocyclops longisetus*)

A. Valdez, J. Montemayor, J. Lewis and
M. I. Abdo de la Parra

Facultad de Ciencias Biológicas,
Universidad Autónoma de Nuevo León,
San Nicolás de los Garza, Mexico

Copepods have been recognized as a potential control agent for *Aedes aegypti* larvae. However, little information about mass rearing of copepods has been reported. A study to better understand the key factors for copepod production was conducted in 1991–92 at the Universidad Autonoma de Nuevo León. Parameters studied were life expectancy, life history, induced quiescence, and use of *Paramecium* in their diet.

Mesocyclops longisetus lived up to 60 days, producing 74–228 progeny, most during the first 10 days of adulthood. At 21–28°C, it took 10–17 days for them to mature to the adult stage. Production of copepods was density-dependent, varying with the number of females. With 25, 50, 75, and 200 females in 1 gallon of water, an average of 1.9, 1.5, 1.0, and 0.3 individuals were produced per day, respectively.

Paramecium caudatum, an alternative diet for copepod mass rearing, was cultured in 6 different food sources: lettuce, beet greens, nonfat powdered milk, wheat, oats, and Maseca® (corn flour).

These diets yielded from 0–1,030 *Paramecium*/ml. The highest production was obtained with 18 g lettuce/liter. Greatest production was obtained from day 4 (856 *Paramecium*/ml) to day 12 (500 *Paramecium*/ml).

After quiescence had been induced, 80–100% survival was achieved with *M. longisetus* that had been stored in a moist environment for 6 weeks. To date, this rearing system has produced more than 100,000 *M. longisetus* for use in local mosquito research projects.

Field releases of *Mesocyclops longisetus* (Copepoda: Cyclopoda) for control of *Aedes aegypti* larvae in 55-gallon metal drums in Monterrey, México (Liberaciones de campo de *Mesocyclops longisetus* para el control de *Aedes aegypti* en tambos metálicos de 55-gal en Monterrey, México)

H. Quiroz-Martinez, C. Solis-Rojas and M. L. Rodríguez-Tovar

Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico

The biological control of *Aedes aegypti* is a new control strategy in Monterrey, Nuevo León, Mexico. After laboratory evaluation of its predatory capacity and because of its high searching capacity, *Mesocyclops longisetus* was selected for evaluation under field conditions.

We studied the impact of this biological control intervention on the larval *Ae. aegypti* population in the "Unidad Pedreras" neighborhood of Monterrey. Three hundred copepods were placed in 60 55-gallon metal drums and 20 55-gallon metal drums had no copepods. For evaluation, samples were taken every 10 days with an aquarium net, which was moved in a circular pattern 5 times in each drum. Samples were placed in small plastic containers to collect and count mosquito larvae and copepods.

The results were analyzed by linear regression, correlation coefficient, and percent of positivity. When a drum appeared without copepods twice, it was reseeded with 300 copepods. If the drum appeared without copepods a second time, it was excluded from the study.

Ten days after copepod application, 48% of the drums were positive but this dropped to 8% 12 weeks after treatment, with an overall mean of 21%. The best statistical value was obtained between copepod settlement and the larval population category, which was the lowest through the samples with a slope $b = -0.44$ and a correlation coefficient $r = -0.42$. The copepods could have kept *Ae. aegypti* 1st and 2nd instar larval populations in the lowest and low categories, that is 0–5 or 6–10 larvae per drum.

Comparative study of susceptibility and residual and air-borne effect of fenitrothion 40 WP on *Anopheles albimanus* in two Nicaraguan villages (Estudio comparativo sobre la susceptibilidad y el efecto residual de fenitrotion 40 WP sobre *Anopheles albimanus* en dos poblados de Nicaragua)

P. Rivera, S. Vargas, M. Picado and M. Mercado

Centro Nacional de Higiene y Epidemiologia, Managua, Nicaragua

The effect of fenitrothion 40 WP 2 mg/m^2 was evaluated during 1991 in 2 localities in 2 different geographic and climatic regions on the northern and Pacific coast of Nicaragua. Susceptibility as well as bioassay tests were performed. The air-borne effect of residual applications was also evaluated. Results indicate that air-borne fenitrothion 40 WP was effective and controlled *Anopheles albimanus* for up to 60 days after application.

Evaluation of the juvenile growth regulator pyriproxyfen (S-31183) against three species of mosquitoes in Panama (Evaluacion del regulador de crecimiento pyriproxyfen [S-31183] contra tres especies de mosquitoes en Panama)

E. Adames and J. Rovira

Universidad de Panama and Ministerio de Salud, Seccíon de Entomología (SNEM), Panama

Ecdysone, a juvenile and cerebral hormone that regulates the cellular functions of growth and metamorphosis, has been isolated and chemically and functionally characterized (Kopec, Wigglesworth, Roller, Williams, Fukuda, and Slama). Subsequently, chemical compounds analogous to ecdysone (Ecdysoides) and juvenile hormones (Juvenoides), known as insect growth regulators (IGRs) or the "third generation of insecticides," were synthesized and evaluated. These compounds are excellent candidates for the control of arthropods; because they act at low concentrations (e.g., ppb), the evolution of resistance is slow in comparison with other insecticides; and they are very safe when used around invertebrates, vertebrates, and humans. Several IGRs have been approved by the U.S. Environmental Protection Agency and include: methoprene, cyromazine, difluomuron, fenoxicarb, and recently pyriproxyfen (S-31183 produced by Sumitomo Chemical, Co. Ltd.).

Field and laboratory studies of pyriproxyfen

were conducted against *Aedes aegypti, Anopheles albimanus,* and *Culex quinquefasciatus* in Panama. Pyriproxyfen (granulated, 0.5% AI) applied at concentrations of 0.025 and 0.050 ppm (mg/liter), inhibited between 82 and 100% of mosquito emergence, with much mortality during the pupal stage and as the adult attempted to emerge.

Under laboratory conditions, 1 and 21 days after the water had been treated with concentrations of 25 and 50 ppb, the emergence inhibition (EI) values were 100% (25 ppb), 100% (50 ppb, one day posttreatment), and 87–96% (50 ppb, 21 days posttreatment). In all cases, the mosquitoes were exposed to these concentrations of pyriproxyfen for 5–6 days.

Similarly, under field conditions, water treated after 1 day and after 21 days (with 5–11 days of exposure), the 25 ppb concentration produced an EI of 98–100% in *Ae. aegypti* and of 100% in *An. albimanus* and *Cx. quinquefasciatus.* The 50 ppb treatment produced 100% EI in *Ae. aegypti* and *Cx. quinquefasciatus* and 95–99% EI in *An. albimanus.*