AGE STRUCTURE AND ABUNDANCE LEVELS IN THE ENTOMOLOGICAL EVALUATION OF AN INSECTICIDE USED IN THE CONTROL OF *ANOPHELES ALBIMANUS* IN SOUTHERN MEXICO

DAVID N. BOWN,[1] MARIO H. RODRÍGUEZ, JUAN I. ARREDONDO-JIMÉNEZ, ENRIQUE G. LOYOLA AND MARÍA DEL CARMEN RODRÍGUEZ

Centro de Investigación de Paludismo, Apartado Postal 537, Tapachula, Chiapas 30700, Mexico

ABSTRACT. Applications of bendiocarb produced a high insecticidal residual effect lasting up to 3 months on the most common indoor house surfaces. No significant decreases in mosquito man-biting rate levels were observed between treated and untreated villages. It was shown that almost equal proportions of intra- and peridomicillary mosquitoes came into contact with the insecticide, indicating that mosquitoes commonly enter houses, rest on treated surfaces and return to bite both indoors and outdoors. Although the insecticide was found to have a significant effect on the percentage parity (interpreted as abundance of older individuals) of intra- and peridomicillary *Anopheles albimanus* mosquitoes, parity recovered and continued a normal cyclic pattern that appeared to be dependent on relative abundance. The proximity of a treated village to an untreated village (1.2 km) can affect the age structure of mosquito populations through shared common resting and breeding sites.

INTRODUCTION

Indoor application of residual insecticides and distribution of antimalarial drugs continue to be the main intervention used in malaria control programs in Mexico and Central America. The primary malaria vector of this region, especially along the coastal plains of Mexico and Central America, is *Anopheles albimanus* Wiedemann. It has a distribution that extends from southern Texas to Venezuela, Colombia and Peru, and commonly occurs throughout the Caribbean (Faran 1980). This species is primarily zoophilic and exophilic (Breeland 1972a) and has been found to have very low infection rates (0.1%) with *Plasmodium vivax* (Grassi and Feletti) (Ramsey et al. 1986). One of the reasons that malaria transmission is thought to be maintained by such a vector is because of high man-biting rates. However, when evaluated by traditional methods, the effect of indoor sprayed insecticides on mosquito relative abundance levels tends to be negligible (Bown et al. 1985). Therefore, the primary objective of indoor spraying is to have a net impact on the probability that the vector will become infective.

When considering the difficulties that confront malaria control programs in the Americas, it is becoming increasingly evident that there is a need to reexamine presently used strategies (house spraying and larviciding) for vector control, including risk factors. This should include a more thorough evaluation of the means to determine the impact of control measures on the vector population. Although the function of the indoor insecticide spray is to decrease longevity of a target vector population, in evaluating this effect it is important to consider what influence other variables may have on seasonal survivorship of this population. Some of these variables are: a) natural fluctuations in abundance, b) changes in the efficacy of the control measure (residual activity), and c) potential for changes in vector behavior.

In the present study, the effects of indoor spraying of an insecticide against *An. albimanus* were monitored over a 30-month period in 2 villages in southern Mexico as part of the national malaria indoor spray program. The main objective was to evaluate how the effect on age structure and relative abundance or man-biting rates reflects the impact of an insecticide on a vector population in a malarious endemic area.

MATERIALS AND METHODS

Study sites: This study was conducted in 3 villages located on the southern Pacific coastal lowlands in the state of Chiapas. El Gancho (pop. 660) consists of 145 houses, which are situated near a banana plantation, about 2.5 km west of the Guatemala border. La Victoria (pop. 600) consists of 205 houses, and is located 2.5 km from the coast and 38 km northwest of El Gancho in an area of mixed agriculture. The third village, Efraín Gutiérrez, (pop. 350) consists of 150 houses and is located inland approximately 1.2 km east of La Victoria (Fig. 1). In general, houses in this area are well ventilated, with palm-thatch roofs and discontinuous walls made of palm poles, split bamboo and, in some cases, cement block.

[1] Author to whom correspondence should be addressed. Present address: PAHO/WHO Project on New Methods for Malaria Control, Plaza España, Edificio Etisa, Guatemala City, Guatemala.

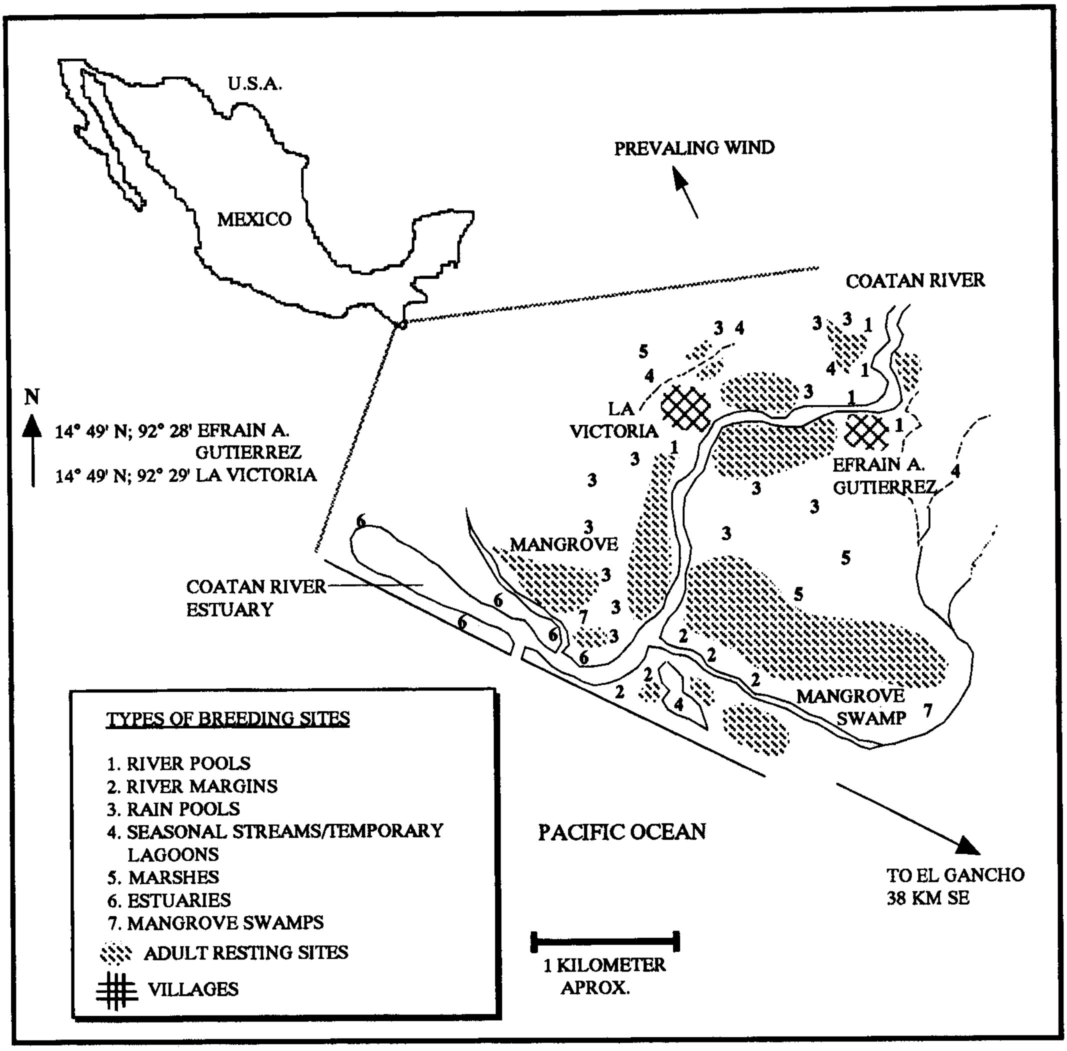

Fig. 1. Map of treated and untreated localities and their proximity to resting and breeding habitats.

These villages were selected because different antimalaria strategies were applied by the national malaria control program and were subject to epidemiological evaluation (results will appear elsewhere). Both insecticide spraying and (trimonthly) mass drug administration (MDA) were applied in El Gancho as antimalaria control measures. La Victoria received only insecticide treatment, while Efraín A. Gutiérrez received only MDA. Houses were sprayed with Ficam® (bendiocarb) water-dispersible powder at a rate of 0.4 g AI/m^2, using conventional hand compression pumps (X-pert®), while MDA consisted of annual trimonthly doses of 1,500 mg of chloroquine and 45 mg of primaquine. El Gancho initially was sprayed with bendiocarb during the second week of August 1986, and La Victoria was first sprayed during the last week of August. Eight spray rounds were carried out at 3- to 4-month intervals by spraying all houses in both villages. Five spray rounds were made during 3 wet seasons (May to October) and 3 during 2 dry seasons. The evaluation included a 30-month period between 1986 and 1988.

Malaria cases were detected in each village through passive surveillance by voluntary health workers. Blood samples were drawn from all febrile cases and examined to confirm diagnosis. A total of 93 malaria cases were detected in the 3 villages, 65 in El Gancho, 14 in la Victoria and 14 in Efraín A. Gutiérrez. In both El Gancho and La Victoria malaria incidence levels remained nearly the same, while in Efraín A. Gutiérrez an increase (from 2 to 12 cases) was recorded during 1988. Although this entomological evaluation included the effect of insecticide spraying on mosquito infection rates, interpretation of these results was confounded by the use of MDA in 2 villages. Therefore, they were not reported.

A mosquito larval ecology survey was conducted from April to December 1988, and included the mapping and characterization of all potential breeding sites for *An. albimanus* around the untreated and treated villages [Efraín A. Gutiérrez and La Victoria (Arredondo-Jiménez 1990[2])]. The mapping was done by walking along variable length transects perpendicular to the river at 500-m intervals from a 4-km distance upstream down to the mouth of the river. At the same time an intensive search for mosquito natural resting sites was carried out by walking along the same transects as was done with the larval survey.

Efficacy of the insecticide on local An. albimanus *populations:* A) Bioassay tests. These tests were conducted on field collected *An. albimanus* twice each month using WHO bioassay kits. Four cones each with 10 blood-fed females were exposed to sprayed wood, palm, pole and cement block wall surfaces for 60 min. Controls were handled in the same manner except that they were exposed to similar untreated wall surfaces in the untreated village. Mosquitoes then were held for 24-h mortality determination.

B) House curtain. Twenty-four trials (12 in El Gancho and 12 in the untreated village), averaging one trial every 10–14 days, were made using 960 mosquitoes in El Gancho and 770 mosquitoes in the untreated village during the peak density period between May and September 1988 (includes 2 spray rounds). These studies evaluated mosquito feeding success, house exiting patterns and indoor mortality rates as a result of exposure to insecticides. To observe movements of mosquitoes in and out of houses with poorly defined walls, the exterior of the houses were encircled with a mosquito curtain from the roof to the ground (Bown et al. 1986a). White sheets were placed on the floor near the inside wall and on the ground under the eaves of the roof to facilitate the collection of dead or moribund mosquitoes. During the experiments (1800–0630 h) the curtain remained down. Starting at 1715 h, the interior of the house was searched for 45 min to remove all live and dead mosquitoes. From 1830 to 2100 h, 4 people collected a minimum of 150 unfed mosquitoes inside other houses from the same village. These mosquitoes were liberated inside the curtained house that contained between 5 and 7 people. During 2200–0600 h, at 1-h intervals, mosquitoes resting between the interior of the curtain and the outside house wall were recaptured and classified as either blood fed or unfed in order to determine the indoor feeding success. Finally, they were placed in plastic cups according to the hour of recapture to evaluate 24-h mortality rates. Dead and moribund mosquitoes found in the corridor between the curtain and the house walls were recovered and mortality rates determined. At 0600 h, a final collection of all indoor resting and dead mosquitoes was made.

Effects of the insecticide on man-biting and age structure: A) Human-bait collections. Mosquitoes collected represent indoor and outdoor hourly contact (1800–2400 h) with man and to some extent indoor treated surfaces. Collections were carried out twice a week over the 30-month period. Two collectors at fixed stations, one sitting inside and the other outside the house, captured landing mosquitoes with an aspirator. The same collectors were rotated every 2 h. Pre- and post-treatment man-biting rates were estimated (no./man/h) and mosquitoes held for 24 h to determine mortality rates. A representative sample of surviving mosquitoes was dissected and examined using the Detinova (1962) technique for age determination.

Data analysis: Statistical comparisons of mortality, feeding success, percentage of exiting mosquitoes from the curtain and parity were carried out by the chi-square test, while differences on mean numbers of man-biting mosquitoes were evaluated by one-way analysis of variance (ANOVA) (Winer 1971).

RESULTS

Bioassays: Mortality varied from one type of sprayed surface to another (Table 1). In El Gancho, 98% mortalities were recorded on all wall surfaces up to 45 days following the first spray. In general, mortalities in both localities tended to decrease to below the 90% level after 60 days. In agreement with earlier studies (Bown et al. 1986b), wood, thatch and bamboo generally sustained a longer residual activity of greater than 75% for 10–15 wk in both localities. Cement tended to lose residual activity at the 75% level or higher after 7 wk. Wood, thatch and bamboo continued to maintain a high residual effect since mortalities below 75% did not occur until 12–15 wk post-spray. The insecticide did not appear to produce a cumulative effect following successive treatments. Less than 10% mortali-

[2] Arredondo-Jiménez, J. I. 1990. Larval ecology of *Anopheles albimanus* Wiedemann (Diptera: Culicidae) in southern Chiapas, Mexico. M.Sc. Thesis. Universidad Autónoma de Nuevo León, México. 70 p.

ties were found on the same surfaces in the unsprayed village.

Curtain collections: Mosquito feeding success on humans (calculated by adding the cumulative percent fed over a 9-h period) was significantly reduced ($P < 0.05$) in the treated village (<25% fed) between May and August (1988) compared with the untreated village (40–45%) between June and August (Fig. 2). Following the first spray in late May, only moderate mortalities were recorded (54%). Highest mortality rates were observed in August (78%) following the second insecticide treatment in late July. There were no significant differences in house exiting patterns between treated and untreated houses, indicating that the mosquitoes were not repelled by the insecticide.

Human-bait collections: As shown in Fig. 3, man biting rates in both treated villages increased nearly 3-fold during the period following the first spray round (to more than 10 mosquitoes per man-hour). Biting rates in the treated localities appeared to follow the same natural seasonal fluctuations as the untreated locality.

Table 1. Results of bioassays on bendiocarb treated surfaces. Exposure time = 60 min.

		Wood		Cement		Thatch		Bamboo	
		≥75%[a]	<75%[b]	≥75%[a]	<75%[b]	≥75%[a]	<75%[b]	≥75%[a]	<75%[b]
El Gancho	Weeks post-spray	10.4	12.1	7.1	7.1	12.1	12.1	12.1	12.1
	No. spray rounds	2	5	3	3	5	5	5	5
La Victoria	Weeks post-spray	12.1	15	4.7	10.4	4.7	15	12.1	15
	No. spray rounds	2	7	5	5	5	7	5	7

[a] Days after treatment ≥75% mortality last observed.
[b] Days after treatment <75% mortality first observed.

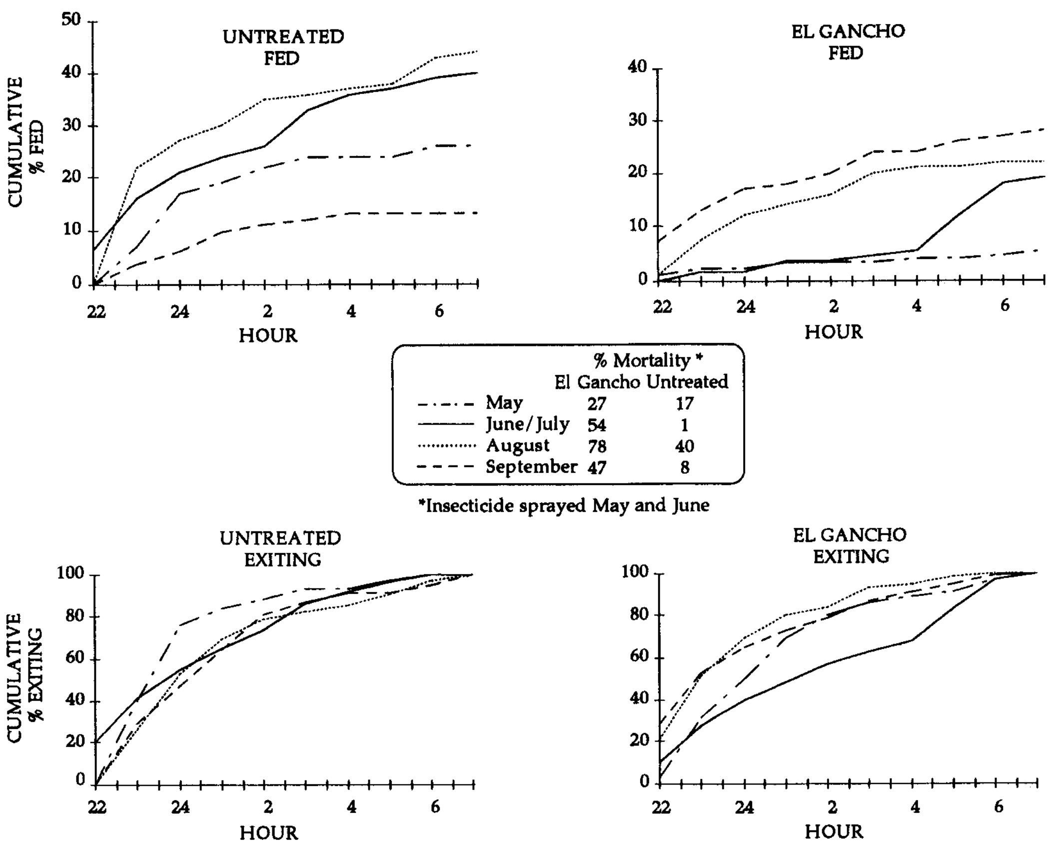

Fig. 2. Results of curtain studies of *Anopheles albimanus* in El Gancho and untreated village.

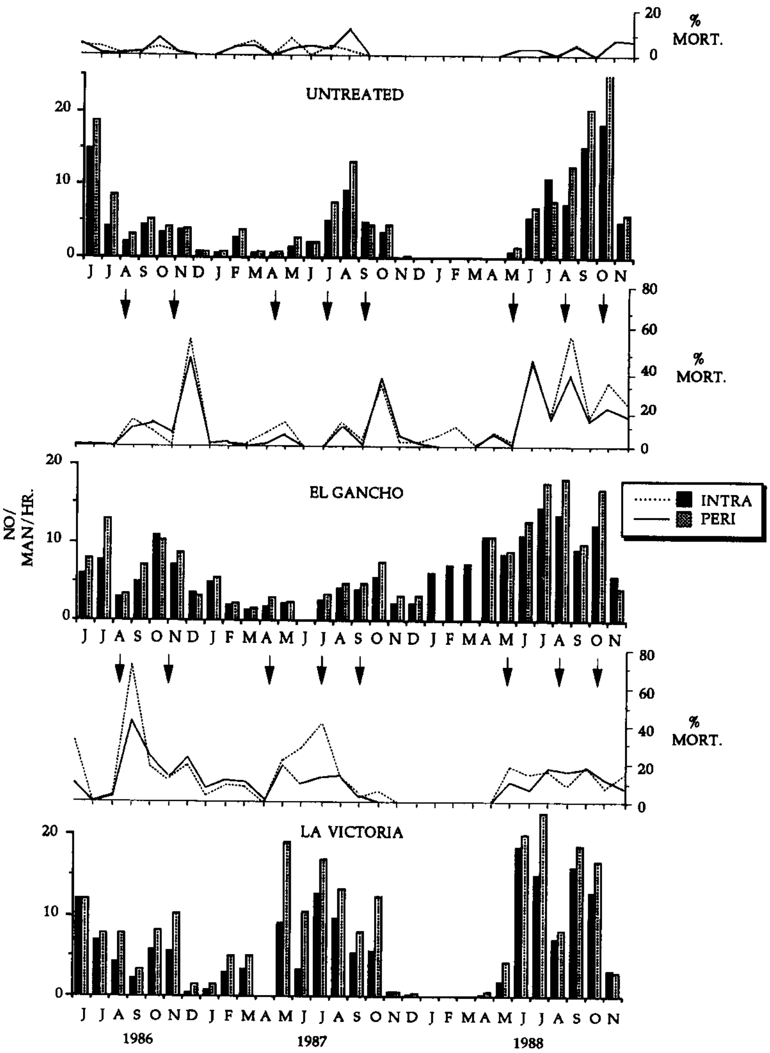

Fig. 3. Number and mortality of *Anopheles albimanus* collected pre- and post-treatment on human bait. Arrows on figure represent insecticide applications.

There were no significant differences between intra- and peridomicillary biting densities ($P > 0.05$). In general, monthly mean man-biting rates were lower (range 0–10.7 bites (b)/man/h) in all 3 villages between November and April 1987–88 and higher (range 1.0–18.8 b/man/h) between May and October, corresponding to the dry and wet seasons, respectively. No significant differences in biting rates were observed between the untreated and treated villages ($P > 0.05$). In addition, overall biting rates increased during the third year (1988).

Human bait mortalities fluctuated between 0 and 73% in the sprayed villages and between 0 and 10% in the untreated village. Higher mortalities were observed during periods of high relative abundance levels, which generally occurred during the wet season. It should be noted that 5 of the 8 spray rounds were carried out during the wet season.

Age grading: To determine the age structure of the mosquito population, 17,170 mosquitoes (more than 5,000 from each village) were dissected during the study. Pre-treatment intra- and peridomicillary parity rates in the 3 localities generally fluctuated between 43 and 65% (Fig. 4). However, following the first insecticide treatments in August, both intra- and peridomicillary parity rates decreased to less than 5% during November and December (1986). During

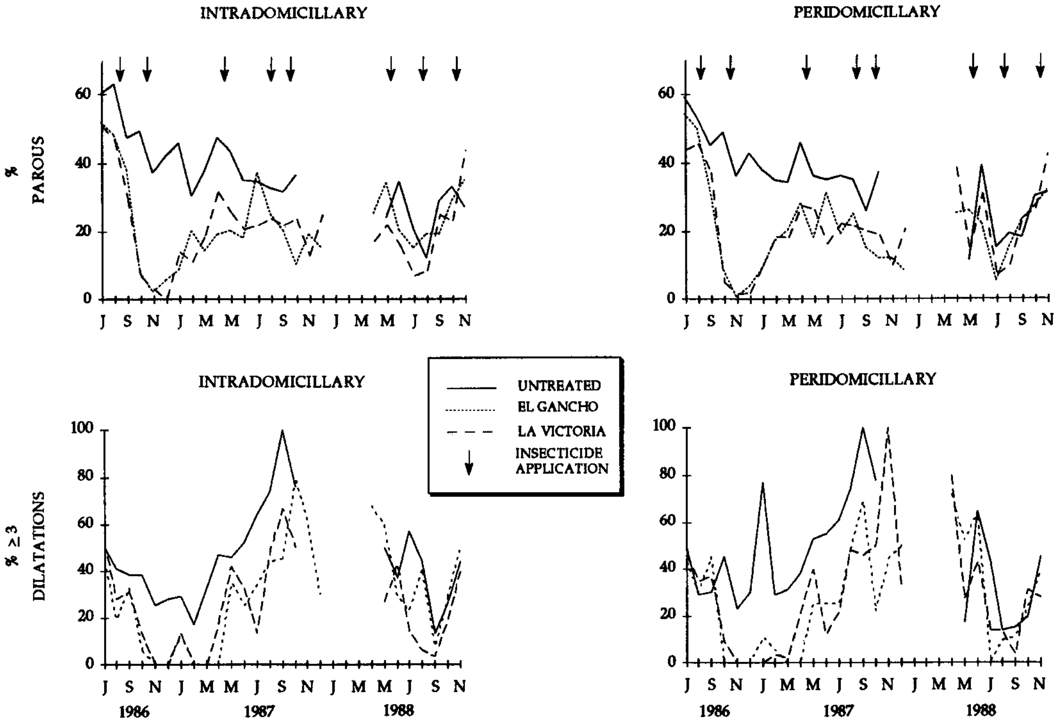

Fig. 4. Pre- and post-treatment changes in age structure of *Anopheles albimanus* collected on human bait.

the same period in the untreated village, parity rates decreased to nearly 40%. Near the onset of the dry season (January 1987), parity in both treatment villages began to increase to between 20 and 30% and continued to increase through the wet season, followed again by a decline to between 10 and 15%, corresponding to the beginning of the dry season of 1988. Parity rates in the untreated village showed a fluctuating but general decline during the same period ranging between 50 and 30%. Because of low numbers between January and March 1988, no mosquitoes were dissected. Significantly lower parity rates ($P < 0.01$) were recorded in the 2 treatment villages (52 to 1%) as compared with the untreated village (62.5 to 26%) during 1986 and 1987. Due to a continued decline in parity in the untreated village, no significant differences were found between treated and untreated villages during 1988, with parity levels in all 3 villages in general remaining between 10 and 30%. This was nearly a 50% overall decrease in parity as compared with pretreatment levels.

The percentage of mosquitoes captured in intra- and peridomicillary with 3 or more ovariole dilatations was calculated from the total number of parous mosquitoes (Fig. 4). Only parous mosquitoes were considered in an attempt to reduce the effect of fluctuations in relative abundance that result from newly emerging nulliparous mosquitoes. Following the first spray round, the percent parous mosquitoes with 3 or more dilatations in the treated villages decreased significantly as compared with the control. During the last 8 months of observation (in 1988), no significant differences were found between treated and untreated villages. In the untreated village normal wet and dry season population fluctuations were observed.

DISCUSSION

The bioassays indicate that surfaces such as wood, bamboo and palm-thatch retained the longest residual insecticide activity. According to Macdonald and Davidson (1953), 75% mortality was the normal criterion of efficacy that an insecticide should achieve against a vector that is both endo- and exophilic. More than 75% mortality was maintained for at least 3 months following insecticide applications. Since a cumulative effect was not noted, a spray cycle of 90 days was considered necessary to maintain acceptable levels of insecticide activity.

Because the WHO bioassay for residual insecticidal activity is widely recognized to be artificial in nature, we included experiments designed to evaluate as much as possible natural mosquito indoor biting and resting behavior. The house curtain was included in the last year of the evaluation to estimate mosquito contact with treated surfaces. Results indicated that *An. albimanus* does not appear to be repelled by the insecticide and remained inside both treated and untreated houses for similar lengths of time. The insecticide was shown to have a significant effect on mortality and feeding success of mosquitoes leaving houses during the night. However, the decline in feeding success was not sustained for more than 45 days after which there was a nearly complete recovery to pre-treatment levels. Overall mortalities decreased to less than 50%, indicating insufficient contact with the insecticide or a reduced residual effect.

Mosquito contact with sprayed surfaces before feeding and the resulting delayed mortality can be observed from the mortality of mosquitoes collected from human bait. The high mortality in peridomicillary collected mosquitoes indicated that mosquitoes first enter houses and then are attracted outside to feed. Mortality of intra- and peridomicillary collected mosquitoes varied, but usually remained less than 40%.

Intra- and peridomicillary human-biting rates increased during the period following the first treatment and increased at least 50% of the time following subsequent treatments in both sprayed villages, indicating that the insecticide did not have a strong impact on mosquito abundance. Mosquito abundance tended to follow annual seasonal variations as determined by rainfall and the availability of breeding habitats. The effect of the indoor spraying in El Gancho and La Victoria was variable. This was especially evident during periods of high population densities. Indoor residual applications were directed only against those mosquitoes that entered houses and rested on interior treated surfaces and therefore was not effective in reducing overall population abundance levels of this species.

Following the first spray round, parity rates in both treated villages decreased to less than 5%. This correlated with an increase in indoor/outdoor man-biting rates in sprayed villages and a decrease in densities in the untreated village. When biting rates decreased naturally during the dry season of 1986–87 (November–May), parity rates gradually increased to a high in the treatment villages of between 20 and 30%, followed by another decrease with the approach of the 1988 dry season. Changes in man-biting rates could in part account for the natural fluctuations of parity rates in treated and the untreated villages. However, the recovery of parous mosquitoes during 1987 following continued insecticide applications suggests a reduction in insecticide effectiveness. Such shifts in age structure are in strong contrast to previous age studies (unpublished data) made in El Gancho, which showed, in the absence of insecticide pressure, very little seasonal variation (40–60% parity rates) despite similar changes in abundance levels.

In an attempt to diminish the input of newly emerged adults in the age structure of the overall population, nulliparous mosquitoes (intra- and peridomicillary) were excluded from the calculation of the proportions of mosquitoes with 3 or more dilatations. As was found in parity rates, a similar sharp decrease in the proportions of multiparous mosquitoes occurred following the first spray in treated localities. We believe that since nulliparous mosquitoes were excluded, these decreases were in part a result of insecticide pressure on older components of the population. As was observed with parous mosquitoes, the same seasonal fluctuations occurred.

In addition to seasonal fluctuations, parity rates demonstrated additional trends, especially in the untreated village, where a gradual decrease occurred following insecticide treatments. By 1988, there were no significant differences found in parity rates in treated and untreated villages 20 months post-spray. Changes in parity in the untreated village closely corresponded to those of the sprayed villages and could not have been brought about by mortalities within the untreated village, as can be seen in Fig. 2. Movement of mosquitoes between treated and untreated villages may explain these observations. The untreated village, Efraín A. Gutiérrez, is in an area isolated from other villages but it is located within 1.2 km of the treated village La Victoria (Fig. 1). The distance between both localities is well within the flight range of *An. albimanus* as determined by Hobbs et al. (1974) and Lowe et al. (1975). Larval surveys conducted between April and December 1988 around Efraín Gutiérrez and La Victoria indicate that seasonal shifts in the availability of breeding sites of *An. albimanus* occur between wet (rain pools, marshes, mangrove swamps and seasonal streams/temporary lagoons) and dry season habitats (estuaries, river margins and river pools) and that most of the breeding sites could provide a common source of mosquitoes for both villages (Arredondo-Jiménez 1990[2]). Because of the proximity of the untreated to the nearest treated village, the indirect effect of the insecticide could most clearly be seen on the age structure in the untreated village during 1988 when compared with pre-treatment levels. Mosquitoes from both villages probably share the same resting and possibly breeding habitats, which would allow mixing of populations to occur. Studies on the diurnal resting habits of *An. albimanus* in El Salvador indicate that it can often be collected near breeding sites (Breeland 1972b). The zoophilic nature of *An. albimanus* and its preference for nonpermanent breeding sites along with the proximity of breeding sites to villages may rule out the possibility of mosquitoes learning a home range and returning to the same village to feed on man, as was found by Charlwood et al. (1988) while studying the dispersal habits of *An. farauti.* Therefore, as a result of population mixing, a gradual decrease in parity in the untreated village could have occurred until no significant differences were found 20 months following the first spray.

This study exemplifies the difficulties in evaluating insecticide spray programs in the control of malaria. Our findings indicate that mosquito man-biting rates are not a suitable parameter to evaluate the efficacy of an indoor spray program of a vector population that is both exophilic and zoophilic. The direct effect on mosquito relative abundance fluctuations as a result of input from newly emerged mosquitoes and the possibility of affecting the age structure of vector population in an untreated area through sharing common resting and breeding sites are important factors that affect the overall evaluation. Changes in the age structure of the vector population can act as an indicator of insecticide pressure when it is directed against the older portion of the vector population. In this case insecticide pressure was able to reduce parity rates, which later returned to normal seasonal cyclic patterns but never returned to pretreatment levels. Mosquito mortalities from 3 different studies indicate diminished insecticide activity that resulted in insufficient mosquito/insecticide contact, causing sublethal intoxication and reduced control. An overall evaluation of these results demonstrates that the effect of the insecticide on mosquito populations should be considered at best insufficient or intermittent. As a result, it allowed cyclic increases in the age structure, which precipitates the potential for malaria transmission to continue.

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