

EFFECT OF INDOOR RESIDUAL SPRAYING OF DDT AND BENDIOCARB ON THE FEEDING PATTERNS OF *ANOPHELES PSEUDOPUNCTIPENNIS* IN MEXICO

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ABSTRACT. Intense and persistent use of DDT for malaria control has increased resistance and induced exophilic behavior of *Anopheles pseudopunctipennis*. An evaluation of bendiocarb and DDT to control this species in Sinaloa, Mexico, showed that, in spite of DDT-resistance, both insecticides produced similar effects. Feeding patterns were analyzed to explain these results. Resting mosquitoes were collected over the dry and wet seasons. Anophelines were tested in an ELISA to determine the source of the meals. The human blood index (HBI) ranged from 3.3 to 6.8% in DDT- and from 12.7 to 26.9% in bendiocarb-sprayed houses. Irritability and repellency in DDT-sprayed houses could explain the reduced HBI. In contrast, bendiocarb produced higher mortality. These effects could have affected different components of the vectorial capacity and similarly reduced malaria.

INTRODUCTION

Anopheles pseudopunctipennis Theobald is considered as one of the principal vectors of malaria in Mexico (Rodríguez and Loyola 1989). Earlier studies on its feeding behavior carried out in Central Mexico, prior to DDT-spraying programs (Vargas et al. 1941), showed that this species readily fed on man and domestic animals. Since then, an intense and persistent use of DDT in vector control programs along with other residual insecticides in agriculture has resulted in resistance and increased exophily (Martínez Palacios and De Zulueta 1963, Elliott 1978).

A comparative study on the use of 2 different residual insecticides, bendiocarb and DDT, to control *An. pseudopunctipennis* in a malarious area in the northwestern state of Sinaloa was carried out between 1985 and 1987. Results showed that, in spite of vector resistance to DDT, both insecticides had similar effects on reducing sporozoite rates and malaria incidence (Loyola et al. 1988, 1991). In an attempt to explain these results, here we analyze the feeding patterns of *An. pseudopunctipennis* recorded during this insecticide evaluation.

Feeding patterns have been defined as the relative frequency of blood taken from different hosts in blood meal samples from a mosquito population in a given place and time (Garrett-Jones et al. 1980). Feeding patterns on human blood are useful indicators in determining the relative importance of *Anopheles* species as vectors of malaria. These patterns can also be useful in the epidemiological assessment of control activities, as a comparative measure of the effect of residual insecticides upon the degree of con-

tact between vector and man (World Health Organization 1963).

MATERIALS AND METHODS

Geographical description of the area: Residual house-spraying of bendiocarb and DDT was employed in contiguous areas (comprising 144 and 94 villages, respectively) in the northwestern state of Sinaloa. One village from each area, with similar ecologic and demographic characteristics, was selected for entomological evaluation. Choice of these villages was based on accessibility, typicality of the people, house type, vector abundance and malaria prevalence. Studies were conducted in the villages of San Miguel de los Orrantia (comprising 326 inhabitants in 76 households) and Palmarito de la Sierra (with 293 inhabitants in 68 households) between April and October 1987. These villages, separated by nearly 75 km, are located approximately 108°W and 25°N at altitudes of 50–300 m, between the coast and the slopes of the western Sierra Madre mountain range, in a region of rolling hills and numerous streams. The climate is hot-semi-humid, with a mean annual temperature of 25°C and an average rainfall of 650 mm. *Plasmodium vivax* is the only malaria species transmitted in the region, and *An. pseudopunctipennis* is considered its primary vector (Rodríguez and Loyola 1989).

Insecticide spraying: Residual indoor spraying with the carbamate insecticide bendiocarb (0.4 g active ingredient (AI)/m²) was carried out in all houses in San Miguel in 2 spray rounds between April and July, whereas DDT (2 g AI/m²) was sprayed in all houses in Palmarito only during April. The residual effect of bendiocarb and DDT have been reported to last for approximately 3 months and 6 months, respectively (Bown et al. 1987, Pant 1988).

Field data collections: Diurnal indoor and out-

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door mosquito collections were carried out from April to October 1987, to include a part of the dry season (April–June) and the whole wet one (July–October). Indoor collections were conducted for 15 min per house (from 0900 to 1100 h) twice per week by each of 2 technicians in a minimum of 25 dwellings selected at random. Mosquitoes were collected resting on walls, furniture and roofs, including those found dead on the floor. This species has been shown to rest primarily outdoors, i.e., in rock crevices, tree holes, bushes and animal burrows (Breeland 1972). Therefore, collections to include these types of resting sites were carried out within the perimeter of the village for 2 h by the same technicians on the same days. Mosquitoes captured were classified as fed or unfed, and by place of collection. From the overall collections only 10% of fed mosquitoes were separated for blood meal analysis, whereas the remainder were used for other studies (insecticide bioassays) (Loyola et al. 1991). The abdomen of fed mosquitoes was separated and smeared onto Whatman No. 2 filter paper. Blood samples were allowed to dry, wrapped with glassine paper and stored at 4°C until processed in the laboratory 3 or 6 months later. A census including the whole human and domestic animal (cows, horses, pigs, dogs and chicken) populations was carried out in each locality in June.

Blood meal identification: Mosquito samples were processed in an ELISA to identify the source of the blood meal using a modification of the technique described by Beier et al. (1988). Samples were eluted overnight at 4°C with 200 μ l of a phosphate buffer saline solution (pH 7.2 PBS). Five μ l of each eluted sample were placed with 50 μ l of coating buffer (sodium bicarbonate 35 mM, sodium carbonate 15 mM, pH 9.6) in 6 wells of a microtiter plate (96 round well/Limbro/Titertek) and incubated for 1 h at room temperature. After blocking unreacted sites with 2.5% dry milk in 7.2 pH PBS, the wells were treated with either one of the following antisera: rabbit anti-IgG H and L chains of human, cow, horse, dog, pig and chicken (Miles Scientific Lab.), followed by a horse-radish peroxidase-conjugated goat serum anti-rabbit IgG (Miles Scientific Lab.). Color was developed using 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma Co.) as a substrate. Blood samples of the species were dried on filter paper and used during tests as positive controls.

Data analysis: Mosquito feeding patterns were assessed by 2 indicators. The human blood index (HBI) is defined as the proportion of freshly engorged anophelines found to contain human blood (World Health Organization 1963). Since

it is usually impossible to obtain an unbiased estimate, this indicator, expressed in percentages, was calculated as the unweighted mean of the HBI from human dwellings and other habitats, as suggested by Garrett-Jones (1964). The forage ratio (FR), an index of host selection which considers the relative availability of hosts, was defined as the weighted mean of engorged mosquitoes with human blood or from other hosts as a function of the availability of that specific host (Hess et al. 1968). A FR of one or near to one indicated an absence of strong host preference; if it was significantly greater than one, it indicated preference for a particular host species, and values less than one indicated avoidance of that species in favor of other hosts. The FR should be considered in general as an indicator of preference (biological significance), whereas the HBI is an indicator of effective contact (epidemiological significance). Since the HBI is influenced by the number of hosts upon which anophelines can feed, if it is adjusted by host availability, the result provides a more accurate indicator of the magnitude of contact between man and vector. Adjustment can be conducted by incorporating the number of available hosts in the locality in the following way: $HBI_{adj} = HBI / (HBI + (ABI(H/A)))$, where HBI is, by definition, the percentage of human blood meals, ABI is the percentage of animal blood meals, H is the percentage of humans in the population, and A is the percentage of animals in the populations. The resulting adjusted HBI was standardized to the expectation for a population which is 50% human and 50% animal.

The chi-square and Fisher's exact tests were used to evaluate the homogeneity of different subsamples of the mosquito population and for 2×2 contingency tables.

RESULTS

A total of 9,480 resting *An. pseudopunctipennis* were collected in the 2 villages (Table 1). Fifty-eight percent (5,501) of all mosquitoes collected were from San Miguel (bendiocarb locality); 33% of these were found resting indoors. In contrast, indoor mosquitoes represented only 5% in Palmarito (DDT locality) ($P < 0.05$). From 47 to 82% of the mosquitoes were bloodfed according to place and season. Also, 56% of all bloodfed mosquitoes were from the bendiocarb locality, 41% being collected indoors. In the DDT locality only 5% were collected indoors ($P < 0.05$). The proportion of bloodfed mosquitoes was roughly the same in the bendiocarb (62% of 5,501 mosquitoes) and the DDT (67% of 3,979 mosquitoes) localities. Resting densities increased between the dry season (April–June,

Table 1. Numbers of indoor and outdoor resting *Anopheles pseudopunctipennis* mosquitoes, according to feeding status and season.

Village	Season ^a	Resting mosquitoes		Bloodfed mosquitoes		Tested mosquitoes ^b	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Bendiocarb locality							
	Dry	339	1,604	251	1,044	24 ^c	115
	Wet	1,517	2,041	1,158	952	204	79
	Total	1,856	3,645	1,409	1,996	228	194
DDT locality							
	Dry	90	1,618	50	953	6	115
	Wet	138	2,133	85	1,589	15	189
	Total	228	3,751	135	2,542	21	304

^a Dry and wet seasons correspond to first and second bendiocarb spray rounds, respectively. Palmarito was sprayed with DDT only once.

^b Other bloodfed mosquitoes were used in other studies.

^c Test of homogeneity between proportions of bloodfed mosquitoes which were indoors and outdoors and their corresponding proportions among those tested ($P > 0.05$).

Table 2. Summary of blood meal analysis of *Anopheles pseudopunctipennis* according to village and collection site.

Host	Bendiocarb locality			DDT locality		
	Indoor ^a	Outdoor	Weighted mean	Indoor	Outdoor	Weighted mean
Human	43.4	4.1	25.4	4.8	6.6	6.5
Animal ^b	56.6	95.9	74.6	95.2	93.4	93.5
Cow	24.6	59.3	40.5	38.1	33.2	33.5
Horse	15.8	22.7	19.0	19.0	35.2	34.2
Pig	6.1	2.6	4.5	23.8	11.5	12.3
Dog	8.8	3.1	6.2	4.8	7.6	7.4
Chicken	1.3	3.1	2.1	9.5	5.9	6.2
Total	100 (228) ^c	100 (194)	100 (422)	100 (21)	100 (304)	100 (325)
Mixed meals	4.4	3.6	4.0	0.0	9.9	9.2
Human blood index ^d		23.8			5.7	

^a Figures expressed as percentage of identified meals within each collection site.

^b All animals combined.

^c No sample.

^d Unweighted mean: (indoor% + outdoor%)/2.

first bendiocarb and only DDT spray round) and the wet season (July–October, second bendiocarb spray round), by 78% in the bendiocarb locality and 33% in the DDT. Only in the bendiocarb locality was this increase more evident in the indoor-resting population. In the DDT locality, the increase in density was accompanied by an increase in the proportion of bloodfeds.

The sample of mosquitoes assayed in the ELISA (12%, range 8.3–17.6%) was in general, proportional to the bloodfed mosquito population distribution according to season and place of collection ("goodness of fit," $P > 0.05$). Identification of blood meal was possible in 82.3% (700) of the 851 mosquitoes tested. All positive controls gave clear reactions with their respective antisera. Multiple feeding was detected in 6.7% (47/700) of the total samples. These individuals were counted twice in the denominator

giving a total of 747 samples. Results from the tests according to village and hosts are summarized in Table 2. Human blood was detected in 25.4% and 6.5% of the samples from the bendiocarb and the DDT localities, respectively ($P < 0.05$). The proportion of human bloodfed mosquitoes was 10 times higher indoors than outdoors in the bendiocarb locality. In contrast, in the DDT locality proportions of human bloodfed mosquitoes were found to be nearly equal indoors and outdoors (4.8 and 6.5%). The overall HBI was 23.8% in the bendiocarb locality and 5.7% in the DDT one. Other major sources of blood were cow and horse, which accounted for 40.5 and 19% of the samples in the bendiocarb locality and 33.5 and 34.2% in the DDT. The other animal blood sources found were pigs, dogs and chicken. Mixed blood meals occurred less frequently in the bendiocarb (3.9%) than in the DDT locality ($P < 0.05$). Nearly 28% (13/47) of

the mixed blood meals contained human blood, and were evenly distributed among different animals.

During the dry season, the weighted mean of samples with human blood were similar in both bendiocarb (5.0%) and DDT (5.8%) localities (Table 3). However, the unweighted mean (HBI) for this season in the bendiocarb locality (11.3%) was higher than in the DDT (3.1%). Significantly more human blood meals were recorded in the bendiocarb locality (weighted mean 35.3%) than in the DDT (6.9%) during the wet season ($P < 0.05$). This can be attributed to the difference between proportions with human blood found indoors, 46.1% in the bendiocarb locality and 6.7% in the DDT ($P < 0.05$). A higher HBI was also estimated in the bendiocarb locality (26.9%) than in the DDT (6.8%) during the rainy season. These indices were more than 2-fold greater than those observed during the dry season.

The results of the census and the FR are given in Table 4. Host densities showed that there were fewer humans than animals. Horses consistently gave the highest FR in both localities (5.03 in the DDT locality and 8.26 in the bendiocarb). Humans had a FR of 1.32 and 0.57 in the bendiocarb and the DDT localities, respectively. According to season, the FR in the bendiocarb locality was lower during the dry (0.26) than during the wet season (1.83), whereas in the DDT locality, the FR for human remained near 0.57 during both seasons. The combined FR for all animals in both villages remained near 1.0 during each season.

The overall HBI adjusted values were 56.6% in the bendiocarb locality and 32% in the DDT localities. The figures for the dry and wet seasons in the bendiocarb locality were 34.8% and 60.6%, respectively, and 24% and 40% in the DDT one. In this case, as man was less available, the adjusted values were higher than the HBI

Table 3. Blood indexes from *Anopheles pseudopunctipennis* with respect to village, collection site and season

Host	Bendiocarb locality			DDT locality		
	Indoor ^a	Outdoor	Weighted mean	Indoor	Outdoor	Weighted mean
Dry season ^b						
Human	20.8	1.7	5.0	0.0	6.1	5.8
Animal	79.2	98.3	95.0	100	93.9	94.2
Total	100 (24) ^c	100 (115)	100 (139)	100 (6)	100 (115)	100 (121)
Mixed meals	8.3	1.7	2.9	0.0	7.8	7.4
Human blood index ^d		11.3			3.1	
Wet season						
Human	46.1	7.6	35.3	6.7	6.9	6.9
Animal	53.9	92.4	64.7	93.3	93.1	93.1
Total	100 (204)	100 (79)	100 (283)	100 (15)	100 (189)	100 (204)
Mixed meals	3.9	6.3	4.6	0.0	11.1	10.3
Human blood index ^d		26.9			6.8	

^a Figures expressed as percentage of identified meals within each collection site.

^b Dry and wet seasons correspond to first and second bendiocarb spray rounds, respectively. Palmarito was sprayed with DDT only once.

^c No mosquitoes tested.

^d Unweighted mean: (Indoor% + Outdoor%)/2.

Table 4. Forage ratios for *Anopheles pseudopunctipennis* during 1987 by village and collection site.

Host	Bendiocarb locality			DDT locality		
	% blood meals	% population	Forage ratio	% blood meals	% population	Forage ratio
Human	25.4	19.3	1.32	6.5	11.4	0.57
Animal ^a	74.6	80.7	0.92	93.5	88.6	1.06
Cow	40.5	9.3	4.35	33.5	23.2	1.44
Horse	19.0	2.3	8.26	34.2	6.8	5.03
Pig	4.5	10.7	0.42	12.3	6.5	1.89
Dog	6.2	8.8	0.70	7.4	7.2	1.03
Chicken	2.1	49.6	0.04	6.2	44.9	0.14
Total no.	422	1,690		325	2,559	

^a All animals combined.

calculated above. However, after adjustment, differences on the HBI between insecticide treatments were reduced. The usefulness of the adjusted HBI is that it may allow comparisons between different situations where the same vector exists. This approach is equivalent to that suggested by Garrett-Jones with the biotopic HBI (Washino and Tempelis 1983), but which is not applicable to the present situation since there were no defined animal shelters in the area.

DISCUSSION

The area where the studies were carried out had been sprayed with DDT for at least 20 years before this was discontinued for 3 years due to anopheline resistance and administrative changes (E. Pérez, Coordinated Public Health Services in the state of Sinaloa, personal communication). Identification of blood meals from *An. pseudopunctipennis* demonstrated that blood-feeding patterns were significantly affected by the type of insecticide sprayed. *Anopheles pseudopunctipennis* from outdoor habitats tended to follow the same feeding patterns from previous studies conducted in central Mexico (Garrett-Jones 1964), which showed that 2% of mosquitoes collected from animal shelters contained human blood whereas 61% were of bovine and equine origin. Although Vargas et al. (1941) reported that 67.7% of samples collected indoors were from man, the present findings showed a lower proportion, ranging from 0 to 6.7% in DDT-sprayed houses and from 20.8 to 46.1% in those collected in bendiocarb-sprayed ones. Numbers of human bloodfed mosquitoes were found to be higher during the wet season in both localities, when mosquito densities were also higher. Other studies have shown an increase of feeding success on hosts during the wet season coinciding with periods of greater mosquito abundance (Washino and Tempelis 1983).

The results also indicated that *An. pseudopunctipennis* tends to be opportunistic in its host-seeking behavior, and that it readily feeds on large mammals. Results from the HBI and the FR used to assess feeding patterns of *An. pseudopunctipennis* on man were similar. The human blood indices were low ($\leq 10\%$) under DDT spraying and moderate (10–50%) with bendiocarb. The forage ratios in the bendiocarb locality were indicative of a random host selection (almost 1), but with a tendency to avoid humans in the DDT locality. A limitation of this study is that no baseline data prior to insecticide spraying are available. However, since villages are very near to each other, differences observed are more likely the result of insecticide spraying.

Insecticides can affect different components of vectorial capacity including the man-biting rate, the man-biting habit (HBI) and longevity (Garrett-Jones and Shidrawi 1969). During the insecticide evaluation, Loyola et al. (1991) found that compared with prespray densities, neither insecticide affected the man-biting frequency. Curtain trap experiments in that study also showed that mosquitoes seeking blood or shelter in DDT-sprayed houses were irritated and driven away by the insecticide. In addition, indoor resting mosquito densities were lower in DDT-sprayed than in bendiocarb sprayed houses due to repellency. Although bendiocarb also had an initial and transient irritant effect, it did not appear to be as effective in reducing the number of indoor resting mosquitoes. Variability in feeding patterns of a given mosquito species can be attributed to host availability (Washino and Tempelis 1983, Nasci 1984). This in part could explain differences between localities, since there were almost twice as many nonhuman hosts in the DDT locality than in the bendiocarb one. The tendency to rest indoors and the proportion of human meals both increased sharply in the bendiocarb locality during the wet season. This suggests that rainy weather tends to drive mosquitoes to shelter indoors, and in consequence they opportunistically fed on humans. The same response to rain was presumably prevented by DDT in Palmarito. This excito-repellent effect of DDT could explain the reduced HBI observed. However, it was observed that bendiocarb produced higher mosquito mortality than DDT. It appears that these 2 different effects of the insecticides on mosquitoes produced similar reductions in malaria incidence in the 2 study villages.

To further investigate this suggestion, vectorial capacity was calculated as described by Garrett-Jones and Shidrawi (1969): $VC = ma^2p / -\log p$. The man-biting habit (a) was derived from $a = HBI/GC$ (Molineaux et al. 1988). Daily survival (p) was estimated from the formula $p = \sqrt[n]{PR}$ as suggested by Davidson (1954). By substitution of a and p, vectorial capacity can be expressed as: $VC = ma(HBI)(PR^{n/GC}) / -\log PR$, where ma is the man-biting rate, HBI the human blood index, PR the parous rate, GC the length of the gonotrophic cycle and n the length of the sporogonic cycle. It was assumed that neither GC which lasts 4 days according to I. Fernández (personal communication) nor n which lasts 9 days according to Macdonald (1957) were affected by the insecticides. The overall unweighted HBI values recorded in the present study were used in the calculations. The value of ma was nearly 12 bites/man/night in the bendiocarb and DDT villages, and the PR were

34.6 and 54.6%, respectively (data from Loyola et al. 1991). The average vectorial capacity obtained in both villages from these parameters (0.247 for bendiocarb and 0.296 for DDT) was similar.

The irritant effect of the residual insecticides is not always counterproductive, and in fact can reduce man-mosquito contact in houses and divert mosquitoes to domestic animals sheltered near human dwellings. A similar effect was observed in Thailand (Goriup and Pull 1988), where DDT-spraying did not reduce the vector longevity, but significantly reduced mosquito resting time in the houses, resulting in a marked behavioral shift in favor of exophily. The irritant effect of DDT, which lasts much longer than the toxic effect, has also been recorded in India (World Health Organization 1986), where the insecticide was reintroduced to take advantage of its irritant effect on a DDT-resistant vector, *An. culicifacies* Giles, and resulted in a reduction of malaria transmission.

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