

## SELECTIVE AND CONVENTIONAL HOUSE-SPRAYING OF DDT AND BENDIOCARB AGAINST *ANOPHELES PSEUDOPUNCTIPENNIS* IN SOUTHERN MEXICO

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**ABSTRACT.** Indoor feeding behaviors and mortalities of *Anopheles pseudopunctipennis* females were evaluated following contact with selective (bands covering mosquitoes' preferred resting areas) and full applications of DDT and bendiocarb on indoor sprayable surfaces. The DDT residues provoked strong avoidance behavior. To a lesser degree, mosquitoes were also repelled by bendiocarb-sprayed surfaces. Because of strong irritancy/repellency, unfed mosquitoes were driven outdoors in proportionally higher numbers. The resting time on selectively or fully DDT-sprayed huts was greatly reduced in comparison to bendiocarb-sprayed huts. Although unfed mosquitoes tended to rest on non-DDT-sprayed surfaces in the selectively treated hut, the man-biting rate was similar with both types of treatments. Unfed mosquitoes were repelled less from selectively bendiocarb-treated surfaces. Similar reductions in postfed resting times were observed on all surfaces suggesting that once fed, mosquitoes rested on sprayed surfaces for shorter intervals of time. Engorged mosquitoes had normal resting behavior (pre- and postspray) within the range of preferred resting heights in both DDT- and bendiocarb-sprayed huts, but the proportion of mosquitoes fed in the DDT-treated huts was lower. Selective spraying of walls was as effective as spraying the complete walls with both insecticides, but DDT was more effective in reducing mosquito-human contact. These studies show that by more effectively targeting vector behavior, a cost-effective alternative to traditional control techniques can be achieved.

**KEY WORDS** DDT, bendiocarb, *Anopheles pseudopunctipennis*, insecticide, control, band spraying

### INTRODUCTION

The importance of *Anopheles albimanus* Wiedemann and *Anopheles pseudopunctipennis* Theobald as principal malaria vectors in Mexico and Central and South America has been well documented (Rodríguez and Loyola 1990, Pan American Health Organization 1991). In Mexico, residual indoor application of DDT has dominated malaria control strategies, having its primary success in areas where *An. albimanus* is responsible for transmission (Rodríguez and Loyola 1990). In areas where *An. pseudopunctipennis* is the principal malaria vector, mosquito feeding patterns are modified in favor of larger outdoor mammals, probably due to direct DDT repellency, despite adequate mortality levels as demonstrated by effective DDT wall bioassays (Loyola et al. 1991). These observations have been documented by Martínez-Palacios and de Zulueta (1964), who concluded that as a result of indoor DDT spraying, this species modified its feeding behavior towards an increased exophily and exophagy. Despite a preponderance of evidence, the relative importance of insecticide repellency and genetic selection through insecticide pressure on exophagy is virtually unstudied.

The intradomicillary resting behavior of *An. albimanus*, the pre- and postfeed indoor resting behavior of *An. pseudopunctipennis*, and their relationship to hosts have been studied in detail in an attempt to clarify population movements before and after females fed on humans (Bown et al. 1993, Casas et al. 1994a). These studies showed that resting sites on walls and ceilings are primarily restricted to narrow bands (< 1 m), and that 87% of the mosquitoes have at least one contact (either with the walls or the ceiling or with both surfaces), along with an overall mean resting time (pre/postfeed) of 8.1 min/landing within this area. Therefore, in regions where the intradomicillary application of residual insecticide is the primary malaria control measure, a high potential for control could be achieved by spraying only the preferred resting sites.

Because DDT continues to be the principal insecticide used in the Mexican malaria control program, the objectives of this study were two-fold: to study the behavior of *An. pseudopunctipennis* in the presence of DDT, to differentiate between the effect of insecticide repellence and exophilic behavior; and to compare the use of DDT with an alternative, less repellent insecticide such as bendiocarb, when applied conventionally, or selectively to preferred resting sites of *An. pseudopunctipennis* females.

### MATERIALS AND METHODS

**Study area:** The study was conducted in an area located along the foothills of southern Chiapas, Mexico (altitude of 660 m), during the dry seasons of 1994 and 1995. The region, hot and semihumid, had a mean temperature of 20.1°C (range 16.5–

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28.0°C), a mean relative humidity of 91.9% (range 60–100%), and a mean annual rainfall of 3,800 mm. Evaluations were conducted in 5 experimental huts that were separated by distances of 200–250 m from each other. The huts were located between 50 and 100 m from the Coatan River and 1.5 km from the nearest community, El Retiro (15°04'39"N, 92°13'20"W). Each hut (4.5 × 3 m and 3 m high) was constructed using similar design and materials (wood siding and corrugated metal roofs) commonly employed in El Retiro. Dichlorodiphenyltrichloroethane and bendiocarb (in 1989) were the only insecticides used in the area over the last 10 years (sprayed every 6 months). Although surrounding villages were sprayed with DDT 6 months before the beginning of the study, the experimental huts were free of insecticide.

**Spray procedure:** Two experimental huts were each sprayed conventionally (full treatment), one with DDT and the other with bendiocarb, whereas 2 received selective DDT and bendiocarb treatments. Dichlorodiphenyltrichloroethane was selected for study because it is inexpensive and is the main insecticide used by the National Malaria Control Program in Mexico. Bendiocarb was selected for study because it has a less irritating effect (Evans 1993). Insecticides were applied using Hudson X-Pert® hand-compression pumps (H. D. Hudson Manufacturing Co., Chicago, IL; HSS 8002-E nozzle, with a regulated discharge of 750 ml/min at a pressure of 40 psi) to spray DDT (75% water dispersible powder [WP]), at a rate of 2.0 g active ingredient (AI)/m<sup>2</sup>. Bendiocarb (80% WP) was sprayed at a rate of 0.4 g AI/m<sup>2</sup>. Two DDT and bendiocarb spray rounds were carried out first on February 15, 1994, in the fully treated huts, and 2 on February 10, 1995, in the fully and selectively treated huts, respectively. Spray operators followed the recommendations of safe insecticide handling as outlined by the World Health Organization (WHO) (1985).

**Selective spray:** The WHO standard technique, as modified by Arredondo-Jiménez et al. (1995), was applied by spraying 2 0.8-m-wide horizontal swaths of insecticide, with the first spraying starting at 0.85 m from the base of walls and extending up to 1.65 m in width, and the 2 starting at the base of inner ceilings and extending 0.8 m upwards. Exterior eaves were not treated. Total spray was completed in 1.6 min.

**Conventional spray:** Following the WHO standard technique, operators, while holding the hose tip 0.45 m from the walls, sprayed 0.8-m-wide vertical swaths each having an overlap not greater than 5%, starting at ceiling level (up to 3 m high) and continuing to the floor. Insecticide was applied to all indoor sprayable surfaces and exterior eaves. Total spray was completed in an average of 3.1 min.

**Insecticide susceptibility tests:** One-day-old male or unfed female *An. pseudopunctipennis* were

exposed to papers impregnated with discriminant dosages of insecticides (4% DDT or 0.1% bendiocarb) in standard susceptibility tubes (WHO 1981). Tests were carried out twice, the first during pretreatment in 1994 and in 1995.

**Insecticide residual effect:** Monthly standard wall bioassays (10 mosquitoes/cone) were conducted to determine the residual effect of both insecticides on sprayed surfaces according to WHO guidelines (1975). Blood-engorged *An. pseudopunctipennis* females used for each test were collected from adjoining nonsprayed areas and randomly assigned to tests on DDT- or bendiocarb-treated surfaces.

**Human bait and indoor resting densities (without curtain-net):** Intra- and peridomicillary biting rates and resting densities were measured for 6 nights (6:00 p.m. to midnight) in all experimental huts before they were sprayed. These observations were repeated for another 6 nights after the huts were sprayed. Two techniques were employed to quantify human-vector contact and preferential resting sites of *An. pseudopunctipennis*. Two technicians conducted human landing collections by positioning one technician inside the hut and one outside for 45 min (technicians were offered weekly chloroquine chemoprophylaxis). Following this interval, both technicians entered the hut and collected all resting mosquitoes on surfaces (walls, ceilings, and other). All mosquitoes were held for 24 h to determine mortality rates. Depending on mosquito densities, 5–10 biting or resting mosquitoes per hour were collected and examined on the following morning to determine parity (Detinova 1962).

**Curtain-net (entrance/exit):** A curtain-net that encircled the exterior of the experimental huts from the ceiling to the floor (Brown et al. 1986) was used to evaluate mosquito movements. During 3 experiments in 1995, 2 technicians collected unfed resting mosquitoes outside of the curtain, between 6:00 and 8:45 p.m. and subsequently dusted them with fluorescent powder (orange or yellow) and released them inside the curtained hut at 9:00 p.m. Two technicians acting as baits were already positioned inside the hut. Marked mosquitoes were found (15 min/h) by use of an ultraviolet lamp and captured while resting on the interior of the curtain. In the interpretation of these data we assumed that marked females on the interior of the net were attempting to leave the hut. At 1:00 a.m. all indoor resting mosquitoes (alive and dead) were collected (Casas et al. 1994b). Feeding status and 24-h mortality of each mosquito were recorded according to time of exit (defined as the time they were collected on the interior net).

**Capture-mark-release (prefeed):** Studies were carried out with the net-encircled experimental houses during the dry season between January and April of 1994 and 1995. Between 6:00 and 8:45 p.m., unfed host-seeking mosquitoes were collected on the exterior of nets at each experimental hut.

Table 1. Insecticide residual effect (% mortality) of DDT and bendiocarb against *Anopheles pseudopunctipennis*.

Period	DDT						Bendiocarb			
	Control		Selective		Conventional		Selective		Conventional	
	Wood	Metal	Wood	Metal	Wood	Metal	Wood	Metal	Wood	Metal
Prespray	—	—	1	1	0	5.4	7.5	5.0	2.5	0
First month postspray	2.9	8.4	62.5	72.5	92.5	92.5	95.0	82.0	100.0	91.3
Second month postspray	2.5	1.3	65.0	72.5	86.5	71.3	91.3	46.3	95.0	75.0
Third month postspray	0	0	—	—	—	—	—	—	95.0	32.5

<sup>1</sup> Hut not yet constructed.

Collected mosquitoes were marked with orange or yellow fluorescent dust. The marked females were then released at 9:00 p.m. at a distance of 3 m from the curtained hut. On the following evening, between 6:00 p.m. and midnight, marked mosquitoes returning to the experimental uncurtained hut to feed were identified using ultraviolet lamps (Casas et al. 1994a). If the mosquitoes entered the hut, their movements were followed for 1 h, recording the number of landings, type of surface, landing height, and resting time. Marked females that landed on humans were allowed to feed and then their movements were followed for 1 h (see below).

**Mark-recapture (postfeed):** The procedure for following postfeed (human contact) mosquitoes was similar to that as prefeed, except that a curtain was not used as a barrier to collect mosquitoes. Between January and March in 1994 and 1995, 3 technicians conducted weekly mark-recapture studies inside experimental huts between 7:00 p.m. and midnight, the time of peak biting activity (Fernández-Salas et al. 1994). One technician served as bait and when an *An. pseudopunctipennis* female landed and engorged, a 2nd technician colored the mosquito with fluorescent powder and followed it, as described above. Mosquitoes were continuously observed for 1 h or until they left the hut (Casas et al. 1994a).

**Data analysis:** Statistical comparisons of the biting and resting mosquitoes, their recapture rates while resting on the exterior of the curtain-net, the proportion of exiting mosquitoes (fed and unfed), and mean resting time on indoor surfaces were evaluated using one-way analysis of variance (ANOVA). Mortality rates were evaluated using one-way ANOVA and a *t*-test following an arcsine transformation (Zar 1984). Reductions in indoor biting due to insecticide treatments with respect to controls (%R) were calculated as follows:  $%R = 1 - [(S_n \times C_o) / (S_o \times C_n)]$  where *S* is the sprayed hut, *C* is the unsprayed hut, *o* is the average number collected before spraying, and *n* is the average number collected immediately after spraying.

## RESULTS

### Insecticide susceptibility tests

*Anopheles pseudopunctipennis* mortality as a result of exposure to DDT was 45% in 1994 and 56%

in 1995. In contrast, susceptibility to bendiocarb was 100% in both evaluations (*n* = 100 mosquitoes per test).

### Insecticide residual effect

Because mortality rates were almost identical following the 1994 and 1995 spray rounds, results are presented together in Table 1.

**Treatment with DDT:** Mortality on wood surfaces (walls) in the fully treated hut remained >80% 2 months after treatment, whereas in the selectively treated hut mortality remained <70% 1 month after insecticide application. Mortality on tin surfaces was slightly higher in the fully treated hut 1 month after spray, but it was <75% 2 months after treatment.

**Treatment with bendiocarb:** Residual activity on wood remained ≥90% in both fully and selectively treated huts for 60 days following treatments and 90 days posttreatment in the fully treated hut. Mortality due to contact with tin remained >75% in the selectively treated hut for only 1 month, whereas in the fully treated hut, mortality ≥75% could be observed for up to 2 months after insecticide spray. Mortality remained <10% on unsprayed surfaces in the untreated hut.

### Human bait and indoor-resting densities

**Treatment with DDT:** Following insecticide treatments, mosquito man-biting rates (in mosquitoes/man/night, hereafter referred to as mmn) in the fully sprayed hut decreased nearly 2-fold indoors (7.8 mmn) but significantly increased more than 4-fold outdoors (61.8 mmn), as compared to the control hut (Table 2). Similarly, following selective application vector-human contact indoors decreased 3-fold (5.3 mmn) but increased from 13.8 to 16.7 mmn outdoors, as compared to the control hut. In the untreated hut, no significant differences were noted in mmn before or after insecticide treatments.

In the fully treated hut, resting densities decreased to 6.8 mmn as compared to the pre- and posttreatment control hut, representing nearly a 5- and 8-fold decrease, respectively. Resting densities in the selectively treated hut decreased even more to nearly 11- to 18-fold from pre- and posttreatment control levels.

Table 2. Mean number ( $\pm$ SE) of *Anopheles pseudopunctipennis* collected landing on human bait either inside or outside experimental huts, and resting on indoor surfaces, and 24-h mortalities. Numbers in parentheses indicate the proportion of mosquitoes collected at each site.

Period	Number of mosquitoes <sup>1</sup>			% mortality		
	Human bait indoor	Human bait outdoor	Indoor resting	Human bait indoor	Human bait outdoor	Indoor resting
Prespray	7.9 $\pm$ 3.4 (16.0%)	8.7 $\pm$ 1.7 (17.7%)	32.7 $\pm$ 11.1 (66.3%)	13.3	13.6	11.8
Postspray						
Control	14.2 $\pm$ 5.9 (17.3%)	13.8 $\pm$ 4.2 (16.7%)	54.3 $\pm$ 36.7 (66.0%)	15.6	12.5	31.4
Selective DDT	5.3 $\pm$ 2.8 (21.2%)	16.7 $\pm$ 3.7 (66.8%)	3.0 $\pm$ 1.7 (12.0%)	27.8	24.6	16.9
Conventional DDT	5.8 $\pm$ 2.4 (7.8%)	61.8 $\pm$ 9.9 (83.1%)	6.8 $\pm$ 4.3 (9.1%)	51.9	26.6	21.4
Selective bendiocarb	30.7 $\pm$ 9.1 (15.3%)	56.2 $\pm$ 11.6 (28.1%)	113.3 $\pm$ 42.8 (56.6%)	25.5	26.1	25.1
Conventional bendiocarb	8.3 $\pm$ 4.7 (11.3%)	49.2 $\pm$ 14.1 (67.2%)	15.7 $\pm$ 8.1 (21.5%)	27.2	38.1	51.8

<sup>1</sup> Number of bites/man/night.

Significantly higher mortality rates were recorded from mosquitoes collected on human bait indoors (51.9%) but no differences were found outdoors (26.6%) following full treatment ( $P > 0.05$ ), as compared to the control hut. Higher mortality rates were also observed from mosquitoes collected on human bait indoors (27.8%) and outdoors (24.6%) following selective treatments. Mortality rates of indoor resting mosquitoes increased from 11.8 to 31.4% in the control hut after insecticide treatments, probably because of contamination with mosquitoes that previously visited the treated huts or to mishandling of this mosquito group. Mortality comparison in indoor resting mosquitoes among treated and untreated huts was therefore not possible. Finally, comparing mortality rates among collection sites, in the conventionally DDT-treated hut higher mortality was observed in mosquitoes collected on human bait indoors with respect to outdoor biting mosquitoes and indoor resting collections ( $P < 0.05$ ). A similar trend was observed in the selectively treated hut, but no statistical differences were observed.

**Treatment with bendiocarb:** Twelve collections were carried out in each of the fully and selectively treated experimental huts during the study period. Although no significant differences were recorded in man-biting rates between the pre- and post-full-treatment control hut, vector-human contact in the conventionally bendiocarb-treated hut significantly increased nearly 6-fold outdoors (49.2 mmn) while remaining nearly the same indoors (8.3 mmn) (Table 2). In the selectively treated hut, nearly a 4-fold increase ( $P < 0.05$ ) occurred indoors (30.7 mmn) in relation to the pretreatment control hut and more than a 3-fold increase occurred in the outdoor mmn (56.2).

Resting densities in the fully treated hut de-

creased from pre- and posttreatment levels by more than 2- and 3-fold, respectively, to 15.7 mmn. However, resting densities increased significantly to 113.3 mmn from pretreatment levels ( $P < 0.05$ ) in the selectively treated hut.

Higher mortality rates were noted from mosquitoes collected on human bait indoors (27.2%) and significant increases occurred outdoors (38.1%) following full treatment ( $P < 0.05$ ), as compared to the control hut. No significant differences in mortality rates were recorded in the selectively treated hut from mosquitoes collected indoors (25.5%) and outdoors (26.1%) following treatments, as compared to control and full bendiocarb application. Mortality of indoor resting mosquitoes within insecticide-treated huts was higher than pretreatment levels (51.8 and 25.1% in the fully and selectively treated huts, respectively). Finally, comparing mortality rates among collection sites, in the fully bendiocarb-treated hut, higher rates were observed in indoor resting mosquitoes with respect to indoor biting mosquitoes ( $P < 0.05$ ), but no differences were observed in relation to outdoor biting mosquitoes. In contrast, almost identical mortalities were recorded in mosquitoes collected at any site in the selectively treated hut.

### Impact on biting behavior

Reductions of 59.2 and 63.0% in indoor mosquito biting were observed in the fully and selectively DDT-sprayed huts, respectively (Table 2). Reductions in indoor biting were observed in the fully bendiocarb-treated hut (41.6%), whereas an increase of 116.2% in biting was calculated for the selectively treated hut.

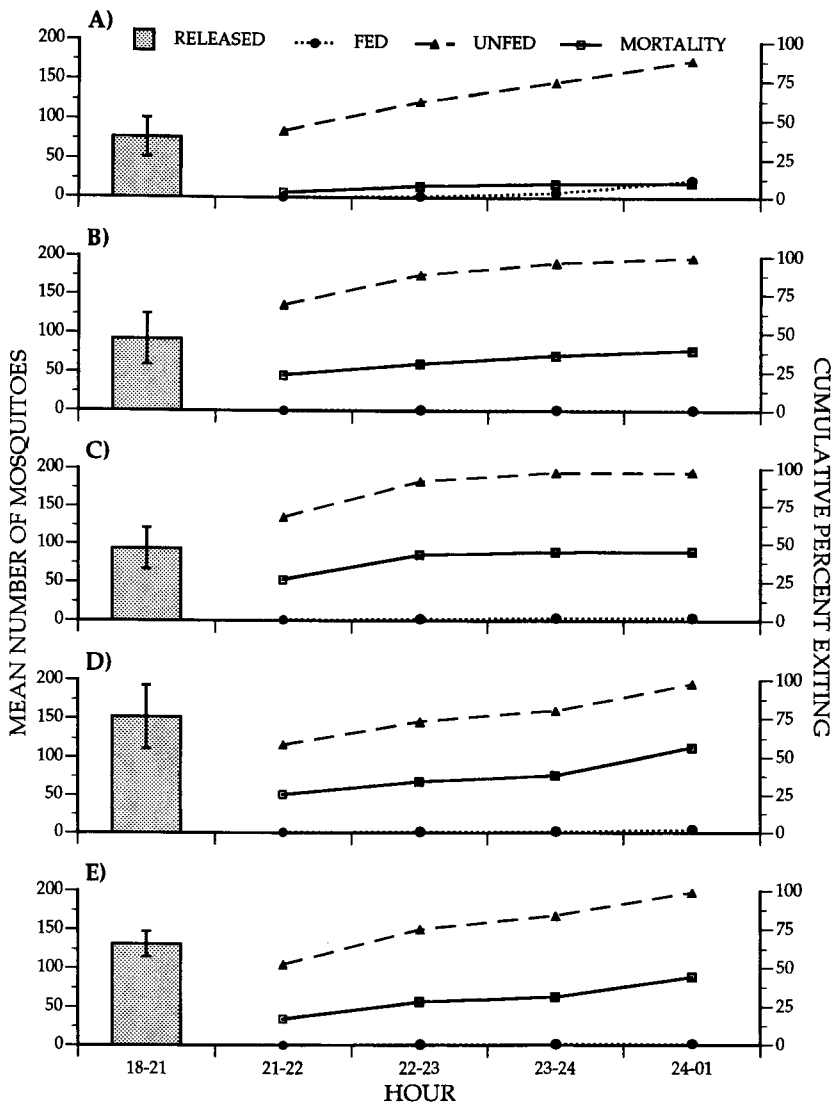


Fig. 1. Feeding and exiting habits and mortality of *Anopheles pseudopunctipennis* during curtain-net experiments in (A) control, (B) DDT conventional, (C) DDT selective, (D) bendiocarb conventional, and (E) bendiocarb selective treatments. Vertical lines represent standard deviations (SD).

#### Curtain-net experiments (entrance/exit)

**Treatment with DDT:** An average of 93 mosquitoes per night was introduced in each experimental hut between 9:00 p.m. and 1:00 a.m., with a recapture rate of marked females indoors between 70 and 76% (Fig. 1). Although significantly higher mortality rates occurred following both DDT treatment types ( $P < 0.05$ ), no differences were observed between full (39.1%) and selective (45.0%) treatments.

More mosquitoes exited the fully treated hut than the control hut (68.9% vs. 43.3%, respectively) during the first hour (9:00–10:00 p.m.) following marking and release ( $P < 0.05$ ). Also, during the

first hour postrelease, no significant differences occurred in the proportions of mosquitoes exiting the hut in the selectively treated hut as compared to the control hut, or exiting the fully treated hut compared to the selectively treated hut. Numbers of mosquitoes exiting between 10:00 p.m. and midnight from the fully and selectively treated huts (96.6 and 99.4%) were significantly greater ( $P < 0.05$ ) than numbers exiting from the control hut (77.7%).

Although few fed mosquitoes exited huts between 9:00 p.m. and midnight, significantly more exited from the control hut (8.3%) than from fully and selectively treated huts (0.0 and 0.3%, respectively) ( $P < 0.05$ ).

**Treatment with bendiocarb:** An average of 142 mosquitoes per night was collected during 3 curtain experiments carried out in each experimental hut, with an average recapture of 76.1%. Significantly higher mortality rates ( $P < 0.05$ ) were observed in full (56.6%) compared to control (<10%) treatments, but no significant difference was observed between control and selective (44.8%) treatments, perhaps due to few observations. Finally, no significant differences were found between full and selective treatments.

During the first hour following mosquito release (9:00–10:00 p.m.), higher mosquito numbers (58.7%,  $P < 0.05$ ) exited the fully treated hut with respect to the control hut (43.3%), but no differences were observed in mosquitoes exiting the selectively treated hut (52.4%) as compared to control hut, or between fully and selectively treated huts (Fig. 1). Cumulative exiting of mosquitoes from fully and selectively treated huts at midnight (81.3 and 84.6%) was not statistically different compared to the control hut (77.7%).

Despite low proportions of fed mosquitoes exiting huts between 9:00 p.m. and midnight, significantly more exited from the control hut (8.3%,  $P < 0.05$ ) compared to the small number exiting from fully and selectively treated huts between midnight and 1:00 a.m. (2.3 and 1.0%, respectively).

#### **Capture-mark-release experiments (prefeed/pretreatment)**

From a total of 2,928 unfed *An. pseudopunctipennis* released outdoors before insecticide treatments, only 50 (1.7%) returned indoors to the 5 experimental huts (full, selective, and control pretreatment). The marked females landed an average of 3 times (range, 0–17). Resting height varied between 86.7 and 119.2 cm on walls and between 250.6 and 274.0 cm on the ceiling, with more than 4 contacts (Fig. 2 and Table 3). Mosquitoes had an overall mean resting time of 25.4 min ( $\pm 23.3$  SD) on walls, followed by 13.7 min ( $\pm 15.6$  SD) on the ceiling and 12.8 min ( $\pm 15.2$  SD) on other surfaces.

#### **Capture-mark-release experiments (prefeed/postselective insecticide spray)**

A total of 203 and 5,449 unfed mosquitoes were released, respectively, in the DDT- and bendiocarb-treated huts. Of these, only 9 (4.4%) and 28 (0.5%) mosquitoes returned the following night and entered band-sprayed houses. Resulting mean indoor-landing frequencies were 2.9 (range 0–13) and 3.3 (range 1–10). Resting heights were <50.0 cm and 69–117 cm on walls, whereas on the ceiling they were between 275.0–290.0 and 233–265 cm (Fig. 2 and Table 3). In the DDT-treated hut, no mosquitoes landed within the sprayed surface on walls and only 50% had contact with the ceiling with the 4th landing. Highest mean resting time for both in-

secticides occurred on other surfaces (4.0–15.0 min and 3.3–15.3 min), followed by 5.3–8.5 min on walls and <1.2 min on the ceiling.

#### **Capture-mark-release experiments (prefeed/postfull treatment)**

From 1,659 and 3,090 females released, respectively, in the DDT- and bendiocarb-treated huts, only 22 (1.3%) and 22 (0.8%) unfed mosquitoes released outdoors returned and entered the fully sprayed huts. Females in the DDT-treated hut had a mean landing frequency of 1.7 (range 0–6), and 4 females (18.2%) had no contact with interior surfaces, whereas inside the bendiocarb-treated hut mean landing frequency was 2.9 (range 0–9). The resting heights on all surfaces varied between 47.0–175.0 and 46.0–115.0 cm on walls, and ranged between 241.7–257.7 and 200.0–260.0 cm on the ceiling (Fig. 2 and Table 3). In the DDT-treated hut, a higher percentage of contacts (64–100%) was recorded on the ceiling. The most prolonged cumulative contact in the DDT-treated hut occurred on walls ( $4.2 \pm 2.2$  min), followed by the ceiling ( $4.1 \pm 5.0$  min) and other surfaces ( $2.6 \pm 2.6$  min), whereas in the bendiocarb-treated hut, the most prolonged cumulative contact occurred on walls ( $16.4 \pm 16.1$  min), followed by other surfaces ( $15.8 \pm 11.2$  min) and the ceiling ( $10.7 \pm 15.6$  min).

#### **Mark-recapture experiments (postfeed/pretreatment)**

Fifty-eight marked females released in the 5 huts, before spraying, produced a mean landing frequency of 3.2 contacts during a 1-h observation period (range 0–7). Mosquitoes rested at heights of 107.5–115.2 cm on walls and 268.8–274.5 cm on the ceiling (Fig. 2 and Table 3). The proportion of mosquitoes contacting these range limits varied between 38 and 64% on both walls and the ceiling. The greatest cumulative contact time occurred on other surfaces ( $27.4 \pm 18.1$  min), followed by walls ( $25.3 \pm 20.5$  min) and the ceiling ( $18.1 \pm 18.3$  min).

#### **Mark-recapture experiments (postfeed/postselective insecticide spray)**

From observations on 30 and 51 fed females, mean landing frequencies of 3.5 (range 0–8) and 3.3 (range 0–13), respectively, were recorded in the selectively DDT- and bendiocarb-treated huts. The resting height on walls varied between 106.5–147.2 and 93.0–115.0 cm, whereas on the ceiling it ranged between 250.0–280.0 and 244.0–278.0 cm (Fig. 2 and Table 3). In the DDT-treated hut, nearly the same number of contacts, as compared with controls, occurred within the spray bands on walls (29–50%), but contacts increased on the ceiling (40–100%), whereas in the bendiocarb-treated hut the proportion of contacts on walls varied between 33 and 53%,

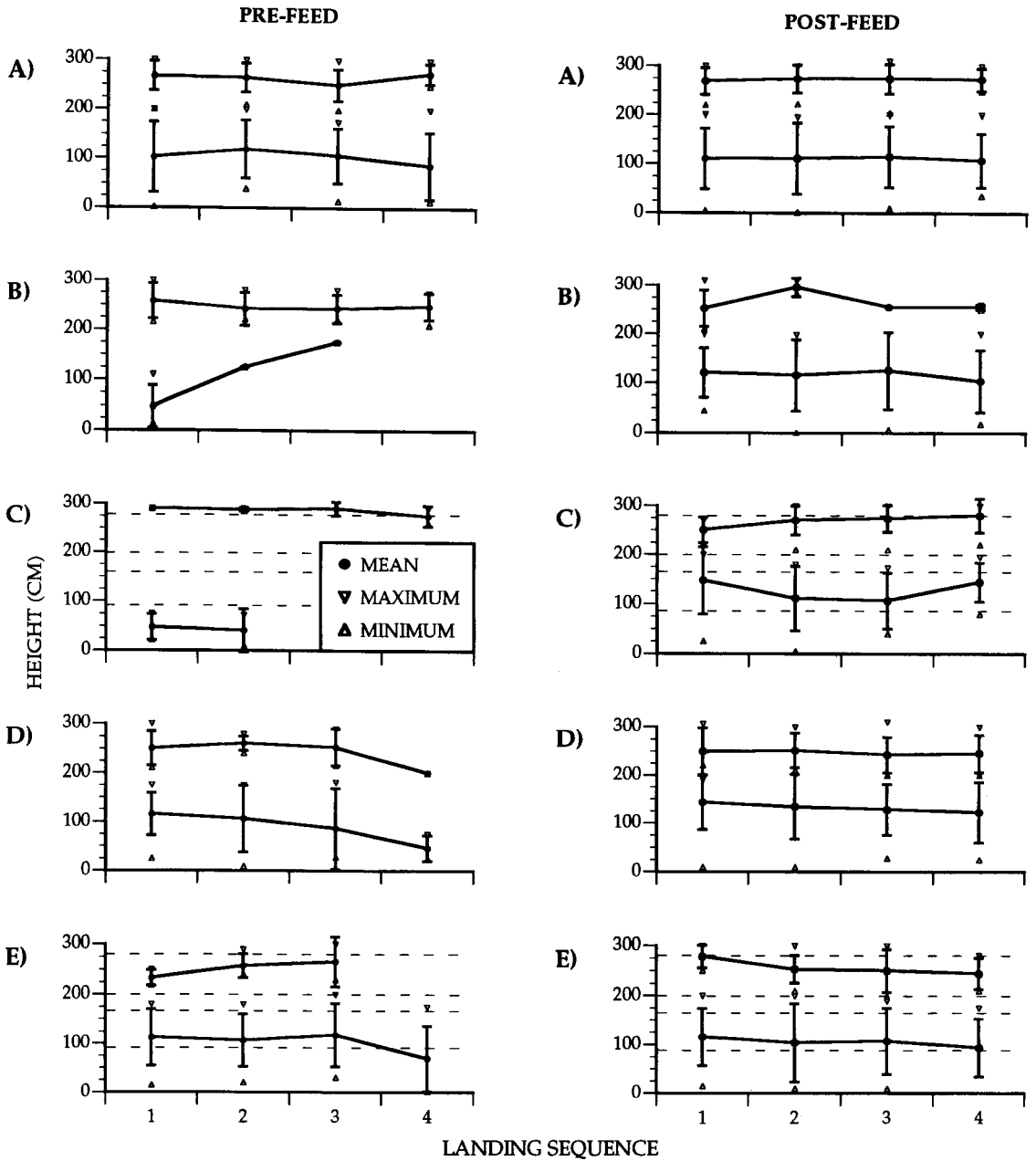


Fig. 2. Prefeed and postfeed mean resting height of *Anopheles pseudopunctipennis* on wall (0–200 cm) and ceiling (200–300 cm): (A) control, (B) DDT conventional, (C) DDT selective, (D) bendiocarb conventional, and (E) bendiocarb selective treatments. Vertical lines represent standard deviations (SD). Broken lines on (C) through (E) indicate insecticide-sprayed bands in the selectively treated houses.

but increased on the ceiling (33–70%). The greatest cumulative contact time in the DDT-treated hut occurred on other surfaces ( $12.7 \pm 15.3$  min), followed by walls ( $5.6 \pm 5.7$  min) and the ceiling ( $3.1 \pm 3.0$  min), whereas in the bendiocarb-treated hut the longest resting time was found on other surfaces ( $21.6 \pm 19.3$  min), followed by walls ( $19.8 \pm 14.8$  min) and the ceiling ( $11.8 \pm 10.4$  min).

#### Mark-recapture experiments (postfeed/postfull insecticide spray)

Seventy-six and 46 fed mosquitoes, respectively, were followed in the fully DDT- and bendiocarb-treated huts with mean landing frequencies of 3.7 (range 0–13) and 3.2 (range 0–9). The resting height in the DDT-treated hut was confined to

Table 3. Indoor resting patterns of *Anopheles pseudopunctipennis* from indoor and outdoor mark-release-recapture experiments in treated and untreated experimental huts.

Surface	Resting variable	Control <sup>1</sup>	DDT		Bendiocarb	
			Selective <sup>2</sup>	Conventional <sup>3</sup>	Selective <sup>4</sup>	Conventional <sup>5</sup>
Prefeed						
Wall	Height (cm)	86.7–119.2	41.0–47.3	47.0–175.0	69.0–117.0	46.0–115.0
	Time/landing (min)	8.2–15.8	5.3–8.5	1.0–2.8	3.7–8.7	6.0–12.7
	Total time	25.4	11.0	4.2	18.7	16.4
Ceiling	Height (cm)	250.6–274.0	275.0–290.0	241.7–257.7	233.0–265.0	200.0–260.0
	Time/landing (min)	2.8–10.7	0.3–1.2	1.4–3.2	3.0–6.0	1.6–17.8
	Total time	13.7	6.2	4.1	6.5	10.7
Other	Height (cm)	0.0–200.0	0.0–200.0	0.0–200.0	0.0–200.0	0.0–200.0
	Time/landing (min)	1.4–8.0	4.0–15.0	1.7–4.0	3.3–15.3	3.0–15.3
	Total time	12.8	15.0	2.6	15.4	15.8
Postfeed						
Wall	Height (cm)	107.5–115.2	106.5–147.2	104.3–125.4	93.0–115.0	122.0–143.0
	Time/landing (min)	6.4–20.8	2.0–7.3	1.8–3.3	3.6–15.4	1.7–8.4
	Total time	25.3	5.6	7.7	19.8	10.0
Ceiling	Height (cm)	268.8–274.5	250.0–280.0	251.7–296.5	244.0–278.0	242.0–251.0
	Time/landing (min)	6.5–10.8	2.4–6.7	1.0–2.5	2.4–9.9	3.7–5.0
	Total time	18.1	3.1	8.4	11.8	5.0
Other	Height (cm)	0.0–200.0	0.0–200.0	0.0–200.0	0.0–200.0	0.0–200.0
	Time/landing (min)	8.0–15.6	4.0–8.7	3.3–5.9	8.8–14.5	3.8–7.7
	Total time	27.4	12.7	10.2	21.6	16.4

<sup>1</sup> Fifty unfed and 50 blood-engorged specimens observed.

<sup>2</sup> Nine unfed and 30 blood-engorged specimens observed.

<sup>3</sup> Twenty-two unfed and 76 blood-engorged specimens observed.

<sup>4</sup> Twenty-eight unfed and 51 blood-engorged specimens observed.

<sup>5</sup> Twenty-six unfed and 46 blood-engorged specimens observed.

104.3–125.4 cm on walls and 251.7–296.5 cm on the ceiling, whereas in the bendiocarb-treated hut it was confined to 122.0–143.0 cm on walls and 242.0–251.0 cm on the ceiling (Fig. 2 and Table 3). In the DDT-treated huts, a low proportion (33–53%) of mosquitoes had contact within 0.85–1.65 m on walls, whereas contacts on the ceiling remained nearly the same (33–70%) as compared with controls. In the bendiocarb-treated hut, reduced contact (29–50%) within the resting range on walls was observed, but contacts on the ceiling increased (40–100%) as compared with controls. The highest cumulative contact time in the DDT-treated hut occurred on other surfaces (10.2 ± 10.3 min), followed by the ceiling (8.4 ± 5.2 min) and walls (7.7 ± 6.7 min), whereas that in the bendiocarb-treated hut occurred on other surfaces (16.4 ± 16.1 min), followed by 10.0 ± 8.9 min on walls and 5.0 ± 4.7 min on the ceiling.

## DISCUSSION

Previous malaria eradication programs and current control strategies share basic technical problems in that they are too expensive, they are difficult to sustain given the extensive areas to be treated, and their coverage is restricted by increasingly limited resources. These shortcomings underscore the need to develop more efficient insecticide application techniques for malaria vector control.

The present data demonstrate that a combination

of the feeding status of the mosquito (pre-/post-feed), the type of insecticide application (selective or full), and the effect (irritant/repellent) produced by the insecticide (DDT or bendiocarb) are complementary factors instrumental in determining mosquito contact with the insecticides. The overall effects were different for DDT and bendiocarb treatments.

Avoidance behavior was clearly demonstrated for unfed mosquitoes in the selectively DDT-treated hut. Mosquitoes reduced contact with DDT by landing above (ceiling) and below (walls) sprayed areas. Although 9 mosquitoes were followed, only one had contact with treated bands, suggesting that unfed mosquitoes avoided contact with sprayed surfaces when unsprayed surfaces were available. In contrast to the modification of mosquito landing patterns in DDT-sprayed huts, unfed mosquitoes made contact with sprayed surfaces within their preferred resting heights (0.85–1.65 m on the walls and from the base of the ceiling 1 m above) in both the fully and selectively bendiocarb-sprayed huts.

Despite changes in resting behavior of unfed populations, engorged females continued to land on their preferred resting sites. Although engorged specimens rested for shorter periods during each landing, nearly 90% had at least 2 contacts within the preferred resting heights on both DDT- and bendiocarb-sprayed walls and ceilings. Other studies, carried out in a village-scale trial with *An. albimanus*, also demonstrated that preferences for rest-



ing sites by engorged females were not altered despite 4 successive bendiocarb spray rounds (Arredondo-Jiménez et al. 1995). However, overall resting time of *An. albimanus* females on sprayed surfaces was reduced.

In the present study, cumulative resting time of unfed *An. pseudopunctipennis* females in DDT-sprayed huts was lower than in the unsprayed control hut. In the selectively sprayed house, resting time was reduced 2-fold and in the fully sprayed hut it was 5-fold less than in the control hut. For bendiocarb, reduced resting time in both the fully and selectively sprayed huts was 1.5-fold or lower than in the control hut. Additionally, reductions in resting time of blood-engorged specimens were also lower in DDT-sprayed huts than in huts sprayed with bendiocarb. We attribute reduced resting times of *An. pseudopunctipennis* females to insecticide irritancy, as previously reported by Evans (1993). Regardless, females still made contact with the insecticides and moderate to low levels of mortality occurred in the study populations.

Excito-repellency of an insecticide can be additionally estimated through a comparison of the reduction of indoor biting patterns following insecticide treatments with respect to pretreatment levels, as well as with indoor/outdoor mortality of human-biting mosquitoes (Bown et al. 1991). In the present study, biting was reduced approximately 60% in both fully and selectively DDT-treated huts, whereas a 40% suppression of feeding was only observed in the fully bendiocarb-sprayed hut. These differences were in part due to the stronger irritant/repellent effect of DDT, and to repellency from the high concentrations of insecticide in the fully bendiocarb-sprayed hut. However, although significantly greater numbers of blood-seeking mosquitoes entered the selectively bendiocarb-sprayed hut, that might be an artifact of sampling.

Furthermore, despite reduced indoor biting in the DDT-sprayed huts, higher mortality rates of biting mosquitoes in the DDT- and bendiocarb-sprayed huts, compared to the unsprayed hut, demonstrate that prior to blood feeding in or outside the hut, a low to moderate proportion of mosquitoes rest on sprayed surfaces long enough to acquire a lethal dosage of insecticide. Prefeeding contact by *An. pseudopunctipennis* on both insecticide-treated surfaces that resulted in moderate mortality in biting mosquitoes was also reported by Loyola et al. (1991).

Opposing results in mosquito mortalities by site of collection and type of insecticide used reflect the contrast between the effect of highly repellent (DDT) and less repellent (bendiocarb) insecticides. Higher mortalities of mosquitoes collected on human bait indoors than of indoor resting mosquitoes in the conventionally DDT-treated hut are perhaps a result of sufficient exposure to treated surfaces prior to feeding. Indoor resting mosquitoes probably were collected before they attempted to bite

technicians, and thus did not have enough lethal contact with treated surfaces. In contrast, in the fully bendiocarb-treated hut, higher mortalities of indoor resting mosquitoes with respect to indoor biting mosquitoes can be explained by the limited repellent effect of bendiocarb.

The presence of insecticide was perceived by some mosquitoes, which tended to land on unsprayed surfaces in the selectively DDT-sprayed hut, although man-biting rates remained unchanged and proportional to those observed in the fully sprayed hut. In contrast, mosquitoes rested on surfaces in the selectively bendiocarb-sprayed hut for similar time spans as in the unsprayed hut, and their resting heights were not modified by the presence of insecticide even when bendiocarb was applied to the entire surface.

The concept of a chemical basis for repellency was previously studied in Africa by Smith and Webley (1968). These authors used dyes and gas chromatography to demonstrate that a steadily decreasing outflow of DDT from a sprayed house had the dual effect of preventing a proportion of *Anopheles gambiae* Giles from entering as well as causing mosquitoes to leave houses earlier. Loyola et al. (1990) likewise observed that irritability and repellency in DDT-sprayed houses reduced indoor biting and resting of *An. pseudopunctipennis*, thereby lowering human blood indices. In the present study, despite lower mosquito densities due to irritancy/repellency, mosquitoes continued to rest on sprayed surfaces, provoking mortalities from both selectively and fully sprayed surfaces, particularly on walls. Because no significant differences occurred in mosquito mortality between selectively or fully DDT- and bendiocarb-sprayed huts (as determined from curtain studies), one can conclude that greater availability of unsprayed surfaces due to selective spraying did not provide additional opportunity for mosquitoes to escape lethal contact.

Although mosquitoes entered DDT- and bendiocarb-treated huts in reduced numbers, greater availability of unsprayed surfaces due to selective spraying did not provide additional opportunity for mosquitoes to escape lethal contact when compared to conventional treatment. Furthermore, despite reduced pre- and postfed resting time, especially in DDT-sprayed huts, sufficient numbers of mosquitoes pursued contact both within and outside preferred resting heights to achieve moderate indoor mortality.

Our curtain studies clearly demonstrated that mosquitoes had at least a 5-fold decrease in feeding tendency in selectively and fully DDT- and bendiocarb-sprayed huts as compared to controls. Loyola et al. (1990), when studying the effect of indoor spraying of DDT and bendiocarb for the control of *An. pseudopunctipennis*, concluded that both insecticides achieved similar efficacy by affecting the feeding patterns (reducing the proportion of mosquitoes feeding indoors on humans due to irritabil-

ity/repellency of DDT) or by increasing mortality rates (due to higher toxicity of bendiocarb). Mosquito exiting patterns in the present study also indicated that between 10:00 and 11:00 p.m., 75% of mosquitoes had attempted to exit from both types of bendiocarb-treated huts, whereas nearly 90% had attempted to exit from DDT-treated huts, indicating stronger excito-repellency of DDT. Only 60% of mosquitoes in the control hut had attempted to exit in the same period. Reduced feeding success, coupled with significantly higher numbers of early exiting mosquitoes from both fully and selectively treated huts (both insecticides) is again due to the excito-repellency of DDT and to the limited repellent effect of bendiocarb. Both effects reduced indoor vector-human contact. Similarly, Fernández-Salas et al. (1993) concluded that the excito-repellency action of DDT was the cause of a decline in proportions of fed outdoor resting *An. pseudopunctipennis*. In the present study, similar proportions of mosquitoes were collected in selectively and fully sprayed huts.

Earlier studies demonstrated that overall mean resting time and height of female *An. pseudopunctipennis* before or after they take a blood meal was within a well-defined range on walls and the ceiling (Casas et al. 1994a). We have confirmed here that effective control can be achieved by using DDT or bendiocarb selectively sprayed on the preferred indoor resting sites of *An. pseudopunctipennis*. In summary, control was attained by forcing strong excito-repellency in mosquitoes, and consequently reducing vector-human contact through earlier exiting patterns, reduced indoor biting, and moderate mortality. Because mosquito mortalities achieved with both insecticides were similar, but excito-repellency was more intense in the DDT- than in the bendiocarb-treated huts, we conclude that DDT is more effective for control of *An. pseudopunctipennis* in southern México, by means of reducing vector-human contact.

Predicting which of the 2 insecticides sprayed selectively on indoor preferred resting sites achieves better malaria control would be difficult. Results demonstrate better performance with band-sprayed DDT, thus providing sound evidence that this insecticide remains valuable and functional. However, local and international pressure to stop the use of DDT in the region and the fact that selectively sprayed bendiocarb is adequate to control the other main malaria vector, *An. albimanus* (Arredondo-Jiménez et al. 1995), may tilt the balance towards this less persistent, but more expensive insecticide. The selective spray technique could as well be 40% less expensive than the cost of full spray, and can be applied nearly 50% faster because of reduced application time and volume of insecticide used (Arredondo-Jiménez et al. 1995). Finally, these results demonstrate that by more effectively targeting vector behavior, a cost-effective

alternative to traditional control techniques can be achieved.

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