# INTRADOMICILLARY PRE- AND POSTFEEDING BEHAVIOR OF ANOPHELES PSEUDOPUNCTIPENNIS OF SOUTHERN MEXICO: IMPLICATIONS FOR MALARIA CONTROL

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ABSTRACT. The intradomicillary pre- and postfeed resting behavior of Anopheles pseudopunctipennis was studied in an experimental house in southern Mexico. During resting periods (both pre-/postfeed) mosquitoes had greater contact (landings) with the inner roof than with the walls and other surfaces. A comparison of mean landing frequency and overall resting time (pre-/postfeed) showed that a greater periodic and prolonged contact occurred prefeed, probably as a result of disturbed activity associated with host movements. Pre-/postfeed resting patterns on walls were limited to a 0.6-0.5-m-wide band, nearly 1 m from the floor, and to a narrower band on the roof, 0.3-0.2 m wide, approximately 2.3 m from the floor, respectively. We calculated that with a band width of 0.8 m on the walls and another band 0.8 m wide on the roof, 87.2% of the mosquitoes had at least one contact with either the wall, the roof, or with both surfaces, along with an overall mean resting time (pre-/postfeed) of 8.1 min/landing. These findings suggest that a high potential for control can be achieved by spraying preferred wall and roof resting sites in this region where the intradomicillary application of residual insecticide is the primary malaria control measure.

#### **INTRODUCTION**

Anopheles pseudopunctipennis Theobald can be found at altitudes between 0 and 2,000 m; however, population abundance is greatest during the dry season at altitudes above 200 m (Hackett 1945) where larvae abound in pools of slow-moving rivers (Savage et al. 1990). The dryseason abundance of An. pseudopunctipennis is an important factor in maintaining transmission in nearly two-thirds of the malarious areas of México, where this species is considered a primary vector of Plasmodium vivax (Rodríguez and Loyola 1989).

Vargas et al. (1941) reported that An. pseudopunctipennis readily fed on humans and domestic animals and Gahan and Payne (1947) reported that it rested indoors after feeding. In part as a result of these and other studies, residual insecticide (DDT) house spraying to reduce longevity of house-entering mosquitoes continues to be the most widely used malaria control measure against An. pseudopunctipennis. However, the irritant effect of DDT (Brown 1958), and the reported increase in exophily and exophagy of An. pseudopunctipennis and Anopheles albimanus Wied. (Trapido 1952, Martínez-Palacios and De Zulueta 1964) are counter indicators for relying on house spraying as the only control strategy.

There is a need to conduct research on other methods, particularly on techniques to improve the effectiveness of insecticides sprayed on house walls (World Health Organization 1963). The objective of this study was to evaluate the preand postfeed indoor resting behavior of *An. pseudopunctipennis* as it is related to more effectively targeting control measures.

## MATERIALS AND METHODS

Study area: The study was carried out in the foothills of southern Chiapas, Mexico, during the dry seasons of 1992 and 1993. The site was located at 660 m above sea level and was surrounded by coffee plantations. Studies were carried out in an experimental house 100 m from the Coatan River and 1.5 km from the nearest community, El Retiro (15°04'39"N, 92°13'20"W). The experimental house (5  $\times$  6 m<sup>2</sup> and 3 m high) was similar to a typical house found in El Retiro (wood siding and corrugated metal roof). This type of construction decreases the ease with which mosquitoes can enter and escape from the house. DDT and bendiocarb (through 1989) were the only insecticides used to spray house walls in the area (sprayed every 6 months) during the last 5 years. Although the village was sprayed with DDT 6 months prior to the beginning of the study, the experimental house was not sprayed and remained insecticide free. The climate can be characterized as hot and semihumid, with a mean

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temperature of 20.3°C (range 15–28°C), a mean relative humidity of 84.8% (range 58–98%), and an average annual rainfall of 3,800 mm. Malaria transmission during the previous year had been as high as 13 cases/1,000 inhabitants.

Capture-mark-release (prefeed): Studies were carried out during the dry season between January and April of 1993. In order to capture unfed mosquitoes, the exterior of the experimental house was encircled with a mosquito curtain from the roof to the ground (Bown et al. 1986). Unfed host-seeking mosquitoes were collected between 1800 and 0600 h while resting on the exterior of the curtain. Then they were dusted with fluorescent powder (Lumogen Yellow®, BASF, Holland, MI) and released outside. On the following evening between 1900 and 2400 h, marked mosquitoes returning to the experimental house to feed were identified using ultraviolet lamps. Three technicians (indoors) were continually searching for mosquitoes entering the house or they identified the mosquitoes already resting on interior surfaces. The number of landings, type of resting surfaces (wall, roof and/or other [furniture, floor, etc.]), and resting time and height were recorded for each marked mosquito. Because of the continuous search, mosquitoes that were first identified while resting were estimated to have had an unevaluated resting time of <5 min. Mosquitoes were continuously observed for 1 h (except for one observation period that continued for >90 min) or until they had contact with human bait (2 technicians).

Mark-recapture (postfeed): The procedure for following postfed (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 1992, 3 technicians conducted weekly mark-recapture studies inside the experimental house during the hours of peak biting activity, between 1900 and 2400 h (Fernández 1992<sup>3</sup>). One technician served as human 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 continually observed for 1 h or until they left the house.

*Data analysis:* Statistical comparisons of pre-/ postfeed resting time on the wall and the roof were evaluated using a *t*-test.

#### RESULTS

Prefeed: Of 1,562 unfed An. pseudopunctipennis released outdoors during 25 experiments, 2.5% returned (39) indoors and of these, 74.4% (29) were first found resting and the remainder were first detected while flying. All 39 mosquitoes were found to have contact with interior surfaces (203 landings) resulting in a mean landing frequency of 5.2 and as many as 18 landings. Of those mosquitoes having at least 4 landings, >45.0% were on wall surfaces (55 contacts), followed by the roof with 41.3% (50 contacts), and other surfaces 13.2% (16 contacts). However, during the last 3 landings (5-7), highest contact (64.3% or 27 contacts) was on the roof, and for an overall total of 7 landings, more mosquitoes (47.2% or 77 contacts) also rested on the roof (Fig. 1).

The most prolonged contact on all surfaces (Fig. 2) occurred during the first landing; on walls  $\bar{x} = 24.1 \pm 28.0$  min (SD), followed by roof  $\bar{x} = 8.7 \pm 11.0$  min and other surfaces  $\bar{x} = 6.7 \pm 1.5$  min. The duration of contact on all surfaces decreased following the first landing to <10 min after the 7th landing. Mosquitoes had longer overall contact through 7 landings; on walls  $\bar{x} = 26.1 \pm 25.7$  min, followed by roof  $\bar{x} = 12.1 \pm 16.6$  min and other surfaces  $\bar{x} = 3.2 \pm 7.7$  min. The overall mean resting time on all surfaces was 13.8 min/landing.

Mosquito contact with wall surfaces through 7 landings was found to have a mean landing height of  $1.3 \pm 0.7$  m (SD) and a mean range of 1-1.6 m (Fig. 3). A mean landing height of  $2.4 \pm 0.3$  m and a mean range of 2.3-2.6 m characterized the height of *An. pseudopunctipennis* landing sites on roof surfaces. Landings on other interior surfaces gradually decreased from 1.3 m to <0.1 m through 7 landings.

*Postfeed:* Twenty-one experiments were carried out during which 124 mosquitoes were followed, resulting in a mean landing frequency of 4.7 for as many as 17 landings. Each mosquito (91.1%) had an estimated mean feeding time of 3.1 min (range 1–8 min).

Although the number of contacts was higher on other surfaces for the first landing with 63 of 120 landings (Fig. 1), for the 6 succeeding landings the numbers of contacts on roof was higher with 42.6% (170 contacts) followed by wall with 31.8% (127 contacts) and other surfaces with 25.6% (102 contacts). The mean resting time of maximum duration was during the first landing on all surfaces with 15.6  $\pm$  12.8 min/landing on walls, 14.0  $\pm$  11.8 min/landing on other surfaces, and 12.3  $\pm$  12.4 min/landing on the roof. For subsequent landings, mean resting time on each surface decreased to less than 6 min/landing. Al-

<sup>&</sup>lt;sup>3</sup> Fernández, S. I. 1992. Bionomics of the primary malaria vector, *Anopheles pseudopunctipennis*, in the Tapachula foothills area of southern Mexico. Ph.D. thesis. Uniformed Services University for Health Sciences, Bethesda, MD.



Fig. 1. Indoor landing frequency of pre- and postfeed Anopheles pseudopunctipennis.

together, mosquitoes had a longer mean resting time (Fig. 2) on other surfaces  $\bar{x} = 15.1 \pm 18.6$ min, followed by wall  $\bar{x} = 11.3 \pm 13.9$  min and roof  $\bar{x} = 10.5 \pm 13.4$  min. The overall mean resting time on all surfaces was 12.3 min/landing.

The magnitude of resting height by type of surface (Fig. 3) was confined to a mean height of  $1.2 \pm 0.6$  m (SD) on wall ( $\bar{x}$  range 0.9–1.4 m),  $2.3 \pm 0.4$  m on roof ( $\bar{x}$  range 2.2–2.4 m), and  $0.2 \pm 0.5$  m on other surfaces ( $\bar{x}$  range 0.1–0.4 m).

*Pre-/postfeed (integrated):* As result of the landing patterns observed from pre-/postfeed intervals, an integrated mean resting pattern was determined to be limited to a band 0.7 m wide (0.9-1.6 m) on walls and to a narrower band 0.4 m wide (2.2-2.6 m) on the roof (Fig. 4). Of those mosquitoes that either rested on walls or the roof

(148 mosquitoes), 42.6% had at least one contact within the 0.7-m band on walls and 49.3% had at least one contact within the 0.4-m band on the roof. Altogether, 73.6% of the mosquitoes had at least one contact either with a wall or the roof, or with both surfaces. It was further calculated that if a band on walls and one on the roof was limited to a width of 0.8 m, 44.6% of the mosquitoes had at least one contact with a wall and 74.3% had at least one contact with the roof. Altogether, 87.2% of the mosquitoes had at least one contact with either a wall or the roof, or with both surfaces. The most prolonged mean contact time on walls and the roof (P < 0.05)within the 0.8-m band occurred prefeed ( $\bar{x} = 11.1$  $\pm$  16.1 min/landing) as compared to postfeed ( $\bar{x}$ =  $6.9 \pm 8.1$  min/landing). An overall mean resting time, pre-/postfeed, was 8.1 min/landing.



† Only one observation.

Fig. 2. Mean resting time of pre- and postfeed Anopheles pseudopunctipennis.

#### DISCUSSION

Anopheles pseudopunctipennis is most abundant during the dry season, and freely feeds outdoors and indoors during the early and late evening on both animals and humans, following which it seeks natural resting sites outdoors as well as inside houses (World Health Organization 1982). However, Loyola et al. (1990) recently reported that feeding patterns can be modified in favor of larger mammals outdoors, as a result of extensive long-term indoor spraying of DDT.

Based on the present study and reports by Edman (1989) and Bown et al. (1993), indoor resting behavior can be viewed as 2 separate components, pre- and postfeed. The first implies host selection and feeding, but as was also observed, includes contact with surfaces before host contact is made. In the 2nd component, after host contact, the vector's orientation changes from feeding to resting and allows a 2nd opportunity for contact with indoor surfaces.

It was found from both pre- and postfeed orientations that mosquitoes rested more frequently on the roof (more contacts) than any other surface. Longest contact was observed on walls during the first 4 landings prefeed. It was also observed that for the first landing postfeed, mosquitoes usually landed on the floor, resulting



Fig. 3. Mean resting height of pre- and postfeed Anopheles pseudopunctipennis. Vertical lines represent standard deviation (SD).

in a higher proportion of contacts with other surfaces. During the 2nd and subsequent landings it was determined that highest contact shifted to the roof, suggesting that mosquitoes began to explore at higher elevations while trying to escape from the house.

A comparison of mean landing frequency and overall resting time (pre-/postfeed) showed that a greater periodic and prolonged contact occurred prefeed, probably as a result of disturbed activity associated with host movements (Service 1971). A further comparison of pre-/postfeed activity demonstrated a decrease in postfeed contact time with interior surfaces as well as an observation that 21.6% of the mosquitoes escaped from the house in less than 60 min. The escaping mosquitoes were found resting on exterior walls and eaves of the house, and on adjacent vegetation (Casas 1993<sup>4</sup>).

It was determined that the pre-/postfeed resting patterns on walls were generally confined to a band 0.6 and 0.5 m wide pre- and postfeed, respectively, and approximately 1 m from the floor. Similarly, pre-/postfeed resting patterns on the roof were limited to an even narrower strip, 0.3 and 0.2 m wide pre- and postfeed, respectively, and approximately 2.3 m from the floor. In earlier studies, resting patterns of *Anopheles albimanus* were found to be similar to those reported herein for *An. pseudopunctipennis*, with a band only 0.15 m higher on walls and at a nearly identical height on the roof but with a range of 0.4 m (Bown et al. 1993). However, when pre- and postfeed mean resting patterns of *An. pseudopunctipennis* were integrated into a single band 0.8-m wide (this width is within the standard swath spraved by the Hudson X-Pert<sup>®</sup>

<sup>&</sup>lt;sup>4</sup> Casas, M. M. 1993. Comportamiento peri e intradomiciliario de *Anopheles pseudopunctipennis* Theobald en el Sur de Chiapas, México. Informe de Servicio Social. Universidad Autónoma Metropolitana, México, D.F.



Fig. 4. Height distribution of landings on walls and roof of pre- and postfeed *Anopheles pseudopunctipennis*. Vertical lines represent standard deviation (SD).

hand-compression pump; World Health Organization 1985) on walls and a similar width on the roof, nearly 90% of the mosquitoes had at least one contact within either of the bands. It was also found that although the mean contact time of mosquitoes resting on the 0.8-m-wide band was significantly longer on the wall and roof prefeed (11.1 min/landing), the mean contact time during both orientation periods (walls and roof) including the overall mean resting time/landing (pre-/postfeed) was well within the range to produce mortality when using DDT (unpublished CIP data).

In a mark-release study by Damar et al. (1980) in Indonesia to determine how control measures can more effectively be used to reduce vector populations, *Anopheles aconitus* Dönitz was found to rest in a similar pattern on walls as in the present study but at a lower height (less than 1 m). Following this, Bang et al. (1981) reduced indoor/outdoor landing and resting rates of the same vector by spraying a single horizontal swath along the lower portion of indoor walls.

In summary, indoor resting behavior was evaluated as 2 essential components (pre-/postfeed); both components afford an opportunity for contact with indoor surfaces. When such contact results in the uptake of insecticide, man/vector contact will be interrupted and vector longevity will be reduced. Accordingly, the findings of this study and those of a previous study (Arredondo-Jiménez et al. 1992) suggest that a high potential for control can be achieved by spraying preferred wall and roof resting sites. When insecticide was sprayed in this manner, it was as effective as traditional spray and required at least 30% less insecticide. In countries that are dependent on the application of residual insecticides, the evaluation of selective spraying of pre- and postfeed preferred resting sites presents an alternative to traditional control techniques. This is especially true in integrated programs with environmental restrictions and an ever increasing competition for resources.

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### **REFERENCES CITED**

- Arredondo-Jiménez, J. I., D. N. Bown, M. H. Rodríguez and E. G. Loyola. 1992. The control of Anopheles albimanus (Diptera: Culicidae) by selective spraying of bendiocarb. Proc. XIII Int. Congr. Trop. Med. Mal. 2:165. Jomtien, Pattaya, Thailand. November 29–December 4, 1992. Mahidol University, Bangkok, Thailand.
- Bang, Y. H., M. Sudomo, R. F. Shaw, C. D. Pradhan, Spratman and G. A. Fleming. 1981. Selective applications of fenithrothion for control of the malaria vector *Anopheles aconitus* in central Java, Indonesia.
  W.H.O. mimeographed document, WHO/VBC 81: 822.
- Bown, D. N., J. R. Ríos, C. Frederickson, G. Del Angel Cabañas and J. F. Méndez. 1986. Use of an exterior curtain-net to evaluate insecticide/mosquito behavior in houses. J. Am. Mosq. Control Assoc. 2:99-101.
- Bown, D. N., M. H. Rodríguez, J. I. Arredondo-Jiménez, E. G. Loyola and M. C. Rodríguez. 1993. Intradomicillary behavior of *Anopheles albimanus* on the coastal plain of southern Mexico: implications for malaria control. J. Am. Mosq. Control Assoc. 9:321-324.
- Brown, A. W. A. 1958. Insecticide resistance in arthropods. W.H.O. Monogr. Ser. 38.
- Damar, T., G. A. Fleming, S. Gandahusada and Y. H. Bang. 1980. Nocturnal indoor resting heights of the malaria vector *Anopheles aconitus* and other anophelines (Diptera: Culicidae) in Central Java, Indonesia. J. Med. Entomol. 18:362–365.
- Edman, J. D. 1989. Are mosquitoes gourmet or gourmand? J. Am. Mosq. Control Assoc. 5:489-497.
- Gahan, J. B. and G. C. Payne. 1947. Control of Anopheles pseudopunctipennis in Mexico with DDT residual sprays applied in buildings. Part I. Am. J. Hyg. 45:123-132.
- Hackett, L. V. 1945. The malaria of the Andean region of South America. Rev. Salubr. Enferm. Trop. 6:239-252.

- Loyola, E. G., M. H. Rodríguez, L. González, J. I. Arredondo, D. N. Bown and M. A. Vaca. 1990. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. J. Am. Mosq. Control Assoc. 6:635-640.
- Martínez-Palacios, A. and J. De Zulueta. 1964. Ethological changes in *Anopheles pseudopunctipennis* in Mexico after prolonged use of DDT. Nature 203: 940-941.
- Rodríguez, M. H. and E. G. Loyola. 1989. Situación epidemiológica actual y perspectivas de la investigación entomológica en México, pp. 15–40. *In:* Memorias del IV Simposio Nacional de Entomología Médica y Veterinaria. Oaxtepec, Mor., México. Sociedad Mexicana de Entomología, Mexico City, Mexico.
- Savage, H. M., E. Rejmankova, J. I. Arredondo-Jiménez, D. R. Roberts and M. H. Rodríguez. 1990. Limnological and botanical characterization of larval habitats for two primary malarial vectors, *Anopheles albimanus* and *Anopheles pseudopunctipennis*, in coastal areas of Chiapas state, Mexico. J. Am. Mosq. Control Assoc. 6:612-620.
- Service, M. W. 1971. The daytime distribution of mosquitoes resting amongst vegetation. J. Med. Entomol. 8:271-278.
- Trapido, H. 1952. Modified response of Anopheles albimanus to DDT residual house spraying in Panama. Am. J. Trop. Med. Hyg. 1:853-861.
- Vargas, L., G. Casis and W. Earle. 1941. Anopheles pseudopunctipennis Theobald, a vector of malaria in Mexico. Am. J. Trop. Med. 21:779–788.
- World Health Organization. 1963. Terminology of malaria and of malaria eradication. W.H.O., Geneva.
- World Health Organization. 1982. Manual on environmental management for mosquito control. With special emphasis on malaria vectors. W.H.O. Offset Publ. 66. W.H.O., Geneva.
- World Health Organization. 1985. Safe use of pesticides. W.H.O. Tech. Rep. Ser. 720.