Behavioral Response of *Anopheles darlingi* to DDT-sprayed House Walls in Amazonia[1]

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The behavioral response of **Anopheles darlingi** *females to spraying of house walls with DDT was studied along the Ituxi River in Amazonas, Brazil, using a house sprayed with 2 g DDT per square meter of wall surface and an untreated house serving as a control.*

It was found that hardly any **An. darlingi** *females entered, exited, or took blood meals inside the treated house after it was sprayed with DDT, and that specimens marked and released inside the house tended to depart immediately. This behavior appears to constitute true repellency rather than contact irritability. Since the typical house in the vicinity of the study site had only two walls, the persistence of malaria in the local area was probably due to home construction practices.*

Spraying of house walls with DDT began in Brazil in 1945–1946 (*1*) and was incorporated into a formal malaria control program in 1959 (*2*). Vigorous house spraying produced extensive malaria-free areas in southern Brazil but was less successful in the Amazon Basin, even though the primary vector, *Anopheles darlingi* Root, continued to be physiologically susceptible to DDT (*2, 3*).

Variability in the endophilic behavior of *An. darlingi* has been proposed as one reason for the limited success of DDT-based control efforts in some regions. Bustamante et al. (*4*) and other investigators (*5*) have observed increased numbers of females resting on the outside walls of DDT-sprayed houses in different parts of Brazil, but the impact of this behavior on malaria transmission has remained unknown. Other researchers have proposed that behavioral avoidance of DDT could be an obstacle to the malaria control effort, but their observations have been either incomplete or preliminary in nature (*6, 7*). It is clear, however, that where DDT has been applied to the walls of well-enclosed houses, the numbers of *An. darlingi* found indoors and the amount of malaria in the community have dropped precipitously (*5, 8–12*).

In 1978 we made a series of three all-night collections in a DDT-sprayed, one-walled house along the Ituxi River in the Brazilian state of Amazonas. We found no detectable differences between *An. darlingi* patterns of host-seeking activity indoors versus outdoors (*13*). We later constructed four-walled houses and conducted uniform collections to elucidate vector behavior within a typical unsprayed native house. After completing preliminary studies from February 1979 to March 1980 (*13*), we studied the influence of DDT-sprayed house walls on vector behavior. The results of this latter study are the subject of this report.

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MATERIALS AND METHODS

A detailed description of the Amazonas study site has been presented previously (*13*). In brief, the site is located at Floresta on the edge of the Ituxi River in the southern part of Amazonas. The Ituxi is a tributary of the larger Purus River that has its headwaters in the states of Acre and Amazonas. Residents of this area generally spend their entire lives on this river system and comprise a widely distributed but stable community. Houses are normally built on "terra firme" (highland areas that are not usually flooded by the river) near the water's edge. The local economy is based on hunting, fishing, and subsistence farming. The houses, constructed on stilts with palm thatch roofs, often have only one or two walls—these latter being made of palm slats,[4] as are the floors. The area is hyperendemic for malaria (*14*) and is characterized by tropical temperatures prevailing throughout distinct wet and dry seasons.

For the studies reported here we constructed two houses of local building materials. The first, built in January 1979, was used for detailed studies on the behavior of *An. darlingi* within the peridomiciliary environment (*13, 15, 16*). Both this and the second house (built in October 1979) were built on stilts approximately 85 cm above the ground, had palm thatch roofs, and had walls and floors made of palm slats. Large openings (greater than 5 cm) between rooms and between the roof and outer walls were screened to reduce mosquito movement. Both houses were located in close proximity to the only two families who lived at Floresta.

Plans were made to spray the first house with 2 grams of DDT (wettable powder formulation) per square meter of wall surface; the second house served as an unsprayed control. After a series of uniform collections was made in both houses, the house to be treated was sprayed; thereafter, three separate series of collections were made—the first immediately, the second two months later, and the third 12 months later. Each series included at least three nights (from 6 p.m. to 7 a.m.) of uniform collections in both houses.

Host-seeking activity was quantified by aspirating mosquitoes, using an oral aspirator, as they landed on the exposed legs of the collectors. These landing collections were made in a uniform manner throughout the study. Paired outdoor-indoor landing collections (using one collector per site) were conducted for 15 minutes per hour. Collectors were rotated between collecting sites, and the two- or three-man teams[5] were switched every six hours. In addition, the teams were rotated between shifts every night. Team members who were not working slept in the houses in order to maintain a normal occupied environment within the test houses. The temperature and humidity were recorded at hourly intervals.

Six to eight window traps per house were placed in windows that were located 2 meters above the ground. These traps were deployed in equal numbers as entry and exit traps, and each trap was emptied at two-hour intervals throughout each collection night.

Blood-engorged *An. darlingi* were collected in the peridomiciliary environment during the early evening and were marked with fluorescent powder. One hundred specimens were then released

[4]A palm slat is a long, thin section split from the outer trunk of a palm tree. Therefore, the slat is flat on the inner surface and rounded on the outer surface. Several slats can be harvested from a single tree.

[5]Consisting of one or two local people and either the first author or Mr. Jose Bento Lima of the University of Brasília (see acknowledgments).

inside each house at 10 p.m. Periodic observations with a long-wave ultraviolet lamp were made following this release to determine how long the released mosquitoes remained in the sprayed and control houses. All these releases were made simultaneously during each series of collections. A more detailed description of the study methods is available in a separate publication (*14*).

The percentage reduction (R) of vector populations in the house sprayed with DDT, as compared to populations in the unsprayed house, was calculated according to a formula used in similar studies carried out in Suriname (*17*), as follows:

$$R\ (\%\ \text{reduction}) = 100\,(1-[(T_n \times C_o)/(T_o \times C_n)])$$

where T represents the treated house, C represents the untreated or control house, o is the number of mosquitoes collected before spraying, and n is the number collected after the house was sprayed. For example, if we had a 75% reduction and the control house contained 100 mosquitoes per night before spraying and 100 per night after spraying, while the treated house contained 100 per night before spraying but only 25 per night after spraying, then

$$R(\%) = 100\{1-[(25 \times 100)/(100 \times 100)]\}$$
$$= 100(1 - 0.25) = 75\%.$$

R was calculated for the series of collections conducted immediately, at two months after treatment, and at 12 months after treatment. We also calculated R values for entrance and exit trap collections made immediately and two months after spraying. No trap data were available for the series of collections conducted 12 months after DDT treatment, because population densities of *An. darlingi* were low and no anophelines were captured.

In addition, during the initial phases of research at the Ituxi River study site we performed two tests for *An. darlingi* susceptibility to DDT using WHO test kits and techniques (*18*). We also assessed the extent to which houses were enclosed along the Ituxi River by counting the number of walls in each of 37 houses within a section of the river that included our study site at Floresta.

RESULTS

The WHO susceptibility tests indicated that Ituxi River populations of *An. darlingi* were susceptible to DDT, the LD_{50} concentration being 0.73% DDT. This level of susceptibility was similar to that found by Rachou et al. in 1957 (*19*), the LD_{50} in that study being 0.71% DDT (*19*).

Our survey of 37 houses found that the average house had 2.2 walls. The kitchen areas, where the family members typically met for the evening meal, had an average of 0.9 walls.

Summary data from the four series of landing collections conducted during this study are presented in Figure 1. Large numbers of *An. darlingi* were collected both outside and inside the two study houses before the house to be treated was sprayed with DDT. This spraying was accomplished at 3 p.m. on 23 March 1980. Collections were performed in both houses and at an outside location that same night. Hardly any specimens were collected in the treated house during the immediate post-spray series of collections, nor were specimens collected in the treated house two months later. In contrast, large numbers of specimens were collected in the control house on the night of 23 March and two months later, although overall population densities declined as the dry season approached. Percentage reductions in biting activity within the treated house, as compared to the control house, were 96.4% for the collections made immediately after treat-

Figure 1. Average numbers of *Anopheles darlingi* captured per night in hourly 10–15 minute landing collections. Each bar represents a series of three or four all-night collections. The collections were made inside the treated house, inside the control house, and outside both houses at the study site along the Ituxi River during the period February 1980–March 1981.

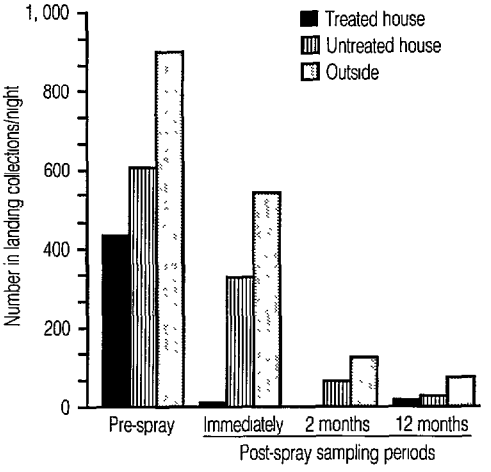
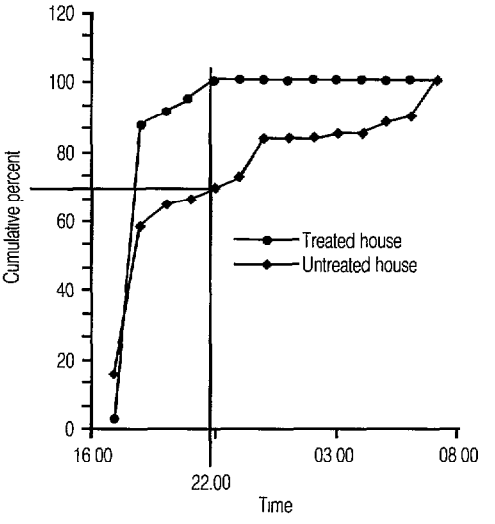


Figure 2. Cumulative distribution of *Anopheles darlingi* captured in landing collections made 12 months after the treated house was sprayed with DDT. The data shown represent the average obtained with three series of all-night collections at each house, each all-night series consisting of hourly 10–15 minute landing collections.



ment and 96.7% for those made two months later.

One year later, in March 1981, the overall population densities of *An. darlingi* were lower than expected, apparently due to unusually low water levels in the Ituxi River (at least up to the time of our visit). Twelve months after the study house's walls were sprayed with DDT, the numbers of *An. darlingi* caught in the treated house were roughly equivalent to the numbers collected in the control house.

Host-seeking activity patterns inside the two study houses during the pre-spray collections were essentially identical, an early evening surge of activity being followed by a gradual decline in the attack rate. However, one year after spraying there was a marked difference in the activity patterns within the treated and control houses (Figure 2). No specimens were captured in landing collections within the treated house after 10 p.m., whereas biting activity continued throughout the night at the control house.

Data from the entrance trap collections made before spraying, immediately after spraying, and two months later are shown graphically in Figure 3. Virtually no specimens were captured in entrance traps at the treated house for two months after it was sprayed with DDT. In contrast, relatively large numbers were collected in entrance traps at the control house. The exit traps at the two locations yielded similar results. Overall, the percentage reductions in numbers collected were 97.9% and 100% in the entrance traps, and 93.7% and 87.9% in the exit traps, for the collections made imme-

Figure 3. Average numbers of *Anopheles darlingi* captured nightly in entrance and exit traps. Each bar represents a series of three or four all-night collections.

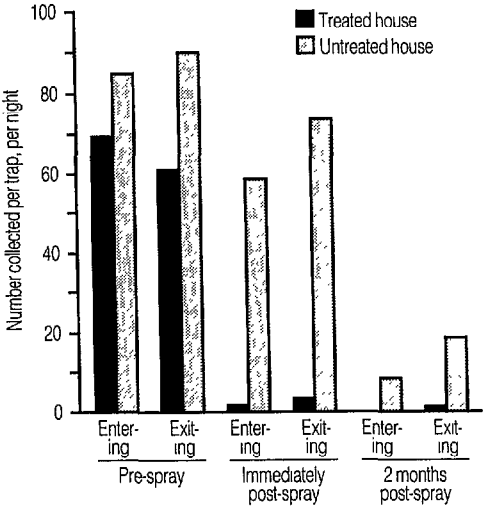
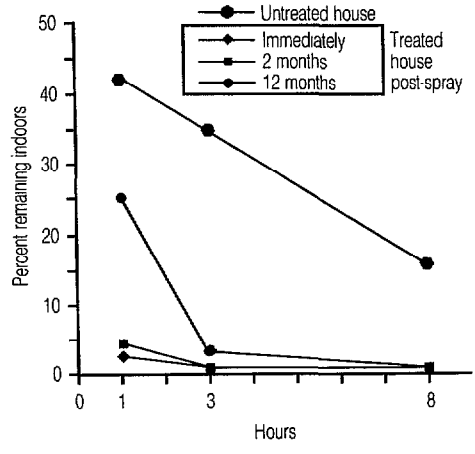


Figure 4. Percentages of *Anopheles darlingi* females remaining indoors one, three, and eight hours after being released into the treated and untreated houses before the treated house was sprayed, immediately thereafter, two months later, and 12 months later. Each of the releases was made at 10 p.m. during the period February 1980–March 1981. Paired releases (one each in the treatment and control houses) of 100 marked females were made in every case. The "untreated" line shows the average data obtained from the one pre-spray release in the treated house and all four releases in the control house.



diately after spraying and two months later, respectively. Chi square tests for two independent samples showed the numbers of females in both exit and entrance traps after spraying to have been significantly higher in the control house than in the treated house ($p < 0.001$ for both tests).

The engorged specimens that were marked and released inside the treated house exited within the first hour after release. Even 12 months after spraying, marked specimens exited the sprayed house quickly (Figure 4). In contrast, many marked specimens remained in the control house throughout the night, following a pattern similar to patterns reported in our earlier studies (*13*).

DISCUSSION

DDT spraying of house walls had a dramatic impact on how Ituxi River populations of *An. darlingi* behaved. In essence, the effect of spraying DDT was similar to dropping a net over the house. For two months after the house was sprayed, hardly any *An. darlingi* females entered, exited, or took blood meals inside the house. Furthermore, specimens marked and released inside the sprayed house departed immediately. All of this contrasted sharply with the high level of activity encountered in the control house.

Historically, behavioral responses of malaria vectors to DDT residues were attributed to contact irritability. However, our earlier observations on euglossine bees that were attracted to DDT residues (*20*) have provided clear evidence that insects possess one or more mechan-

isms for noncontact detection of such residues.

Van Thiel (*21*) has used the term "definite repellent effect I" to define a response to an insecticide that prevents mosquitoes from entering a sprayed house. In discussing this subject, Georghiou (*22*) states that insecticide "irritability" results from physical contact with the insecticide, while the detection and avoidance of an insecticide without physical contact constitutes "repellency." The results of our study clearly demonstrate "definite repellent effect I," or, as defined by Georghiou, insecticide repellency. Interestingly, Van Thiel (*21*) speculated that there was only one known report—according to which a squad of malaria control spraymen obtained relief from man-biting *An. darlingi* by sprinkling DDT under their beds at night—that could possibly be classified as "definite repellent effect I."

Our study also provided insights into the effects of house enclosure and the time elapsed since spraying upon the impact of DDT repellency. We found that DDT treatment of a one-walled house had no detectable effect on the numbers of *An. darlingi* collected or their pattern of host-seeking activity indoors versus outdoors—as indicated by paired indoor and outdoor collections (*13*). In contrast, a recently sprayed, well-enclosed house afforded almost complete protection against host-seeking populations, the percentage reductions in indoor biting activity being 96.4% immediately after spraying and 96.7% two months later. One year later, in the same house, we found that the anophelines entered and took blood meals but exited quickly. This latter finding is based on the observation that while specimens were captured biting in the control house throughout the night, none were captured in the DDT-sprayed house after 10 p.m. Also, many of the marked specimens released in the control house remained inside until sunrise, whereas specimens released in the DDT-sprayed house exited within three hours.

Our earlier studies had indicated that movement of host-seeking *An. darlingi* populations into houses occurs almost exclusively during the early evening, and that indoor biting activity peaks in the early evening (*13*). The study reported here has shown that one year after the treated house was sprayed with DDT, *An. darlingi* populations entered the house early; then, whether they fed or not, they exited quickly to escape the DDT residues. Consequently, there were no host-seeking populations inside the treated house after 10 p.m.

An. darlingi populations from different geographic areas present great biologic diversity. In contrast to our observation that indoor activity ceased almost completely in a treated house, data from Suriname indicate that DDT house spraying reduced *An. darlingi* biting activity by only 20.3% (*17*). More recent studies in Suriname have demonstrated that such house spraying produced a 32% reduction in entry rates, a 43.6% reduction in feeding success, and a 24-hour mortality of 95% among those mosquitoes that entered the sprayed house (*23*).

Despite this diversity, excito-repellency test results have revealed fairly consistent behavior by *An. darlingi* from different geographic areas. Specifically, *An. darlingi* populations collected north of Manaus, Brazil, have demonstrated escape patterns very similar to those exhibited by *An. darlingi* populations tested at the Ituxi River site (*7, 15*). Unfortunately, the investigation north of Manaus included no comparable studies on the behavioral responses of *An. darlingi* to sprayed houses.

No irritability to DDT, as assessed by the number of flights of individual mosquitoes during a 15-minute exposure to

2% DDT on filter paper, was observed with natural populations of *An. darlingi* in Colombia (24). Interestingly, Colombia is also the source of the first *An. darlingi* populations reported to be physiologically resistant to DDT (25).

The reasons for marked differences between behavioral responses and physiologic susceptibility to DDT residues in different areas are unknown. Different patterns of DDT use by the different areas' malaria control programs do not seem responsible, because DDT has been used commonly in Suriname, Brazil's Amazon Basin, and Colombia. However, it might be desirable to quantify the significance of behavioral differences by means of carefully executed studies using excito-repellency test boxes, performing physiologic susceptibility tests, and making detailed observations about vector behavior in sprayed and unsprayed houses in geographically distinct locales. Such studies might also prove extremely valuable in defining the true impact of DDT-sprayed house walls on malaria transmission.

CONCLUSION

We found that 2 g of DDT/m^2 sprayed on the wall surfaces of reasonably well-enclosed houses strongly repelled host-seeking *An. darlingi* populations; such spraying should provide considerable protection against malaria transmission to residents along the Ituxi River. A phenomenon reported by other investigators (6)—of *An. darlingi* females entering a well-enclosed, sprayed house, taking blood meals, and exiting the house without making contact with the DDT-treated surfaces—did not occur during the first two months after spraying. However, this type of behavior did begin to occur sometime between two and 12 months after spraying.

The typical house along the river was constructed with only two walls. This high prevalence of poorly enclosed houses appears to be the primary reason for the persistence of malaria transmission in spite of a well-disciplined DDT spraying program.

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