Preliminary Field Testing of a Long-Lasting Insecticide-Treated Hammock Against *Anopheles gambiae* and *Mansonia* spp. (Diptera: Culicidae) in West Africa

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ABSTRACT The efficacy of an experimental long-lasting insecticide-treated hammock (LLIH) with a long-lasting treated net used as a blanket and made of the same fabric (polyethylene) was tested in a concrete block experimental hut, against the malaria vector *Anopheles gambiae* s.l. and the arbovirus vectors and nuisance mosquitoes *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald). The LLIH was treated with the pyrethroid insecticide permethrin. It was evaluated concurrently with ignited mosquito coils over 20 successive weeks. In total, 2,227 mosquitoes (130 *An. gambiae* and 2,097 *Mansonia* spp.) corresponding to 27.8 mosquitoes per trap-night were collected in the untreated hut (control). The repellent effect of both coils and LLIH significantly reduced the number of mosquitoes entering the huts (35–60%). There was no significant difference between LLIH and mosquito coils in blood-feeding inhibition (93–97%) or in mortality (88–98%). The LLIH is more cost-effective and user-friendly than mosquito coils, which need to be replaced nightly to protect people sleeping indoors from mosquito bites. The effects of LLIH on exophagic vectors also need to be investigated because most people that sleep in hammocks are outdoors.

RÉSUMÉ L'efficacité d'un hamac expérimental muni d'une couverture en moustiquaire, tous deux en polyéthylène imprégné d'un insecticide pyréthrinoïde (perméthrine) et à longue durée d'efficacité, a été testée dans une case expérimentale en parpaings de ciment contre des populations naturelles de *Anopheles gambiae* s.l., vecteur de paludisme, et de *Mansonia africana* (Theobald) et *Mansonia uniformis* (Theobald), moustiques nuisants et vecteur d'arboviroses. Le hamac a été évalué pendant 20 semaines consécutives en comparaison avec des tortillons insecticides. 2,227 moustiques (130 *An. gambiae* et 2,097 *Mansonia* spp.), soit 27,8 moustiques par nuit de capture ont été collectés dans la case non traitée (témoin). L'effet répulsif des tortillons comme celui du hamac ont diminué significativement le taux d'entrée des moustiques dans les cases (de 35 à 60%). Le pourcentage d'inhibition du taux de piqûres a été élevé, tant pour les tortillons que le hamac (de 93 à 97%) ainsi que la mortalité (de 88 à 98%). En protégeant durablement les personnes dormant à l'intérieur des habitations, il apparaît que les hamacs imprégnés sont plus pratiques que les tortillons dans la mesure où ces derniers doivent être remplacés chaque nuit. Il serait intéressant d'évaluer l'efficacité de ces hamacs imprégnés contre les vecteurs exophages dans la mesure où la plupart des gens dormant spontanément dans des hamacs les utilisent à l'extérieur.

KEY WORDS insecticide-treated hammock, permethrin, field testing, malaria vectors, pest mosquitoes

The large-scale use of insecticide-treated nets is a major component of the overall strategy of malaria control (Lines 1996). Over the last decade, long-lasting, wash-resistant pyrethroid-treated nets that remain active for their entire average life span without any retreatment have been promoted to overcome the irregular retreatment of nets. Due to recent developments in bioactive fiber technology, other long-lasting insecticide-treated materials, such as curtains, tents, clothing, blankets, and plastic sheeting have been tested as potential alternatives to mosquito nets. However, they proved to be effective only in addition to mosquito nets or in limited epidemiological or cultural settings (Rowland et al. 1999, Guillet 2001).

People using hammocks while sleeping outdoors or indoors also could benefit from this technology against

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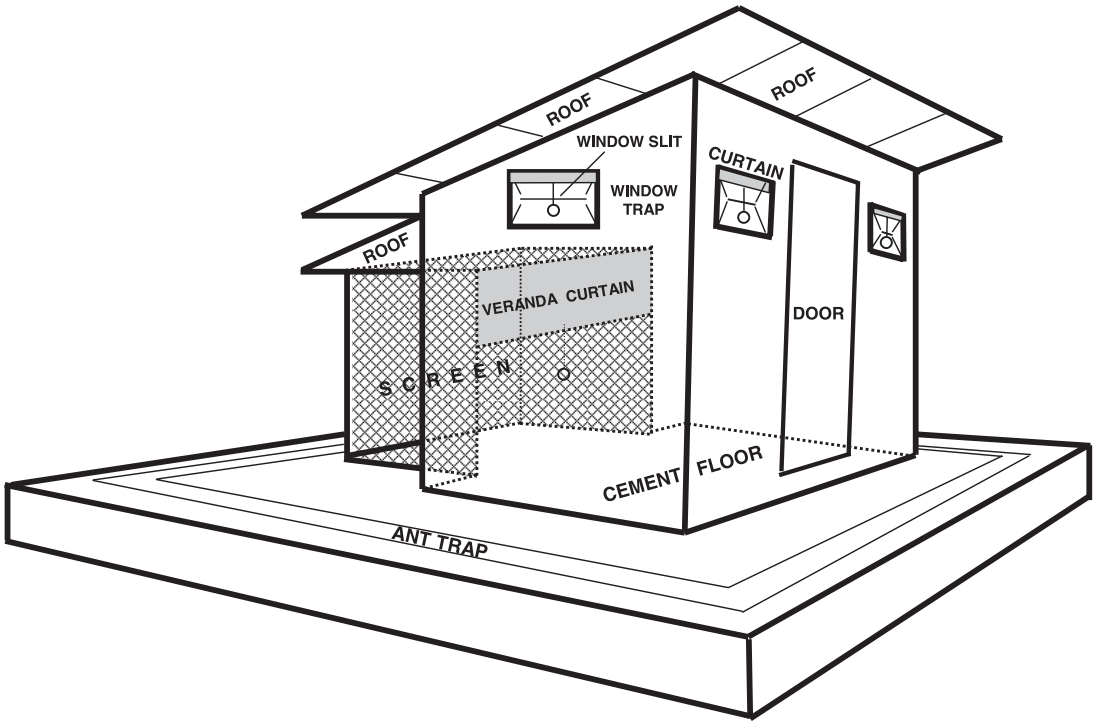

Fig. 1. Design of the experimental huts commonly used in West Africa for small-scale field trials against mosquitoes (credit J.-M.H.).

exophagic and endophagic malaria vectors. We report the mosquitocidal efficacy of a long-lasting insecticide-treated hammock (LLIH) for use against the arbovirus vectors and nuisance mosquitoes *Mansonia* spp. and the malaria vector *Anopheles gambiae* s.l. under well-controlled conditions in experimental huts.

Materials and Methods

The LLIH used for this study was an experimental hammock made of open mesh high-density polyethylene multilayer film containing 2% (wt:wt) permethrin (pyrethroid insecticide) at a *cis-trans* ratio ranging from 50:50 to 30:70 (Sumitomo Chemical Co. Tokyo, Japan). It included a net, also treated with permethrin and made of the same fabric used as a blanket. At this dosage and isomer ratio, permethrin incorporated into polyethylene filaments follows the interim specifications recently proposed by the World Health Organization (WHO 2006).

The test was conducted in a savannah environment in Malanville, northern Benin, West Africa, near irrigated rice fields and ponds. Biting mosquitoes morphologically identified to species were *An. gambiae*, the major malaria vector in Africa (Hougard et al. 2002), and *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald) (Laurence 1960), both major nuisance mosquitoes and vectors of arboviruses. Ninety-five percent of *An. gambiae*, identified to species and molecular forms by polymerase chain reaction (PCR) analysis, were *Anopheles gambiae* s.s. (M molecular form) and 5% were *Anopheles arabiensis* (Patton). The very few mosquito species collected other than *An. gambiae* s.l., *Ma. africana*, and *Ma. uniformis* were discarded.

Construction of experimental huts (Fig. 1) consisted of concrete brick walls, with a corrugated metal roof, a ceiling of thick polyethylene sheeting, and a light gray concrete floor (to facilitate mosquito collection) surrounded by a water-filled channel to prevent entry of ants (Darriet et al. 2002). Mosquito access was via four window slits on three sides and a large screened veranda on the fourth side (the back wall of each hut). The window entry slits were constructed from sheets of metal, fixed at an angle to create a funnel with a 1-cm-wide gap. Mosquitoes flew upward to enter through the gap and downward to exit; this greatly limited the number of mosquitoes exiting from the aperture, and it enabled the majority of entering mosquitoes to be accounted for. The veranda trap was 2 m in length, 1.5 m in width, and 1.5 m in height, and it was made of polyethylene sheeting and screening mesh. Mosquitoes could only exit into the verandah, which was shut at dawn by lowering a curtain separating the sleeping room from the veranda.

Before the trial, differences in hut attractiveness to mosquitoes were tested by putting one volunteer in each hut during 12 nights without any treatment. Three huts were treated as follows: 1) an untreated control, 2) one commercially available mosquito

coil containing 0.2% *d*-allethrin burning from 2000 to 0600 hours, and 3) a LLIH hung from opposing walls ≈50 cm above the floor. Human volunteers in the control and mosquito coil huts slept on a cotton mattress with a bed sheet. This trial received the ethical clearance of the national authority of Bénin (through the Ministry of Health). The volunteers were informed on the objectives of this study and signed an informed consent form including a detailed human use protocol. The three volunteers entered the hut at 2000 hours and left 0600 hours. To avoid contamination with *d*-allethrin, the treatments were not rotated among huts.

Adult volunteers slept in the huts during the entire night, four nights per week. They were rotated randomly among each of the huts nightly during the study. In the morning, dead mosquitoes were collected from the floor of the hut and the veranda trap; resting mosquitoes were collected from the walls and roof of the hut and the veranda trap by using mouth aspirators. Mosquitoes in each hut were scored as dead or alive and fed or unfed. Live mosquitoes were put into small cups, and they were provided with a sugar solution for 24 h to assess delayed mortality.

Blood-feeding inhibition (BFI) and mortality resulting from the use of LLIH and mosquito coils were compared. Mortality was assessed during the morning collection (immediate mortality) and 24 h later (delayed mortality). The percentage of mortality is derived from the number of dead mosquitoes compared with the total number in the hut. The percentage of blood feeding is derived from the number of blood-fed female mosquitoes compared with the total number in the hut. The BFI is the reduction of blood feeding between a treated hut and the control hut.

The ability of the treatment to deter mosquito entry (deterrence/repellency) and to induce mosquito exiting into the veranda (exophily) also was assessed because these parameters may contribute to a better interpretation of the two main outcomes. The entry rate is the total number of female mosquitoes found in the hut and veranda trap. The difference of entry rates between a treated hut and a control hut allowed an estimation of deterrence/repellency due to treatment. The percentage of exophily is the proportion of female mosquitoes found in the veranda compared with the total number present in the hut. The reduction in exit rates between the treated huts and the control hut (representing natural exophily) allowed an estimation of the induced exophily.

The proportional data were analyzed using logistic regression (XLSTAT 2006 software, Addinsoft, Paris, France). Because of the non-normality of the numbers of mosquitoes collected from each hut, these data were analyzed using the nonparametric Mann–Whitney *U*-test for each pair of huts. The differences in hut attractiveness were globally compared using a Kruskal–Wallis nonparametric test.

Results

Interhut Attractiveness to Mosquitoes. Preliminary catches without any treatment were conducted for 12 trap-nights in the three experimental huts, 2 wk before starting the trial. In total, 763,679, and 719 *An. gambiae* and *Mansonia* spp. were collected in huts 1, 2, and 3, respectively, corresponding to an average of 63.6, 56.6, and 59.9 mosquitoes per trap-night. There was no significant difference in hut-to-hut attractiveness ($K = 0.68$, df = 2, $P = 0.71$ [> 0.05]; $n = 36$).

Efficacy of LLIH in Experimental Huts. Untreated huts and huts with a LLIH or mosquito coils were evaluated for 20 wk (80 trap-nights), from November 2005 to March 2006 (Table 1). During this period, 2,227 mosquitoes in total (130 *An. gambiae* and 2,097 *Mansonia* spp.), corresponding to 27.8 mosquitoes per trap-night, were collected in the untreated hut (control). Both coils and LLIH repelled a significant percentage of mosquitoes from entering the hut (35% with *An. gambiae* [$P < 0.05$], 60% with *Mansonia* spp. [$P < 0.0001$]). Coils were significantly more repellent than LLIH with *Mansonia* spp. ($P < 0.0001$) but not with *An. gambiae*, although a higher percentage was repelled ($P = 0.201$). For *An. gambiae*, exophily did not differ significantly between huts, including the control hut ($P > 0.8$). For *Mansonia* spp., a slight but significant induced exophily ($P < 0.0001$) was observed with coils (20.4%). Blood feeding in the treated huts was significantly lower than in the control hut ($P < 0.0001$), but it did not differ between the two treatments or with mosquito genus ($P > 0.05$). The percentage of BFI was high with the two treatments for both mosquito genera (93–97%). Immediate and delayed mortality in the treated huts was significantly higher than in the control hut ($P < 0.0001$). No significant difference between treatment and species was noted, excepted for *Mansonia* spp. between coils and LLIH ($P < 0.0001$). Percentages of immediate mortality, which varied from 82 to 94% according to treatments and mosquito species, were slightly lower than those of delayed mortality (88–98%).

Discussion

The lower number of mosquitoes caught per trap-night in the control hut during the trial (28.3, i.e., half the average number recorded during the preliminary catches) is a confirmation of the importance of the presence of stagnant water on mosquito densities. In late October 2005, in the absence of irrigation and sufficient rains, larval numbers dropped rapidly in the surrounding rice fields and ponds, resulting in a progressive decline in adult mosquito population until reaching a plateau due to the maintenance of perennial breeding sites.

Because deterrence was relatively high, the values given by proportions of blood-fed mosquitoes in the treatment huts may underestimate the full personal protective effect. Thus, it is better to assess this parameter relative to the control, according to the following formula: $100 \times (B_c - B_t)/B_c$, where B_c is the

Table 1. Efficacy of mosquito coils and LLIHs in experimental huts, against *An. gambiae* s.l. and *Mansonia* spp. (80 trap-nights, 7 Nov. 2005–20 Mar. 2006)

	Control (%)		Mosquito coil (%)		LLIH (%)	
	An. gambiae	*Mansonia* spp.[a]	*An. gambiae*	*Mansonia* spp.	*An. gambiae*	*Mansonia* spp.
No. mosquitoes[b]	130a	2,097c	65b	842d	85b	1,265e
Deterrence[c]			50.00	59.85	34.62	39.68
Exophily[d]	62.31 (81) [53.7–70.2]a	53.31 (1,118) [51.2–55.4]b,c	63.08 (41) [50.8–73.]a,b,c	62.83 (529) [59.5–66.0]a	63.53 (54) [52.8–73.0]a,b	52.09 (659) [49.3–54.8]c
Induced exophily.[e]			N.S.	20.38	N.S.	N.S.
Blood fed[f]	97.69 (127) [93.1–99.3]a	97.62 (2,047) [96.9–98.2]a	6.15 (4) [2.3–15.3]b	2.97 (25) [2.0–4.4]b	7.06 (6) [3.2–14.8]b	3.64 (46) [2.7–4.8]b
BFI[g]			93.70	96.96	92.77	96.27
Mortality (immediate)[h]	1.54 (2) [0.4–5.9]a	0.86 (18) [0.5–1.4]a	87.69 (56) [77.3–93.7]b,c	82.30 (693) [79.6–84.7]b	92.94 (79) [85.2–96.8]b,c	93.52 (1,183) [92.0–94.7]c
Mortality (delayed)	1.54 (2) [0.4–5.9]a	1.24 (26) [0.8–1.8]a	95.38 (62) [86.6–98.5]b,c	87.89 (740) [85.5–89.9]b	97.65 (83) [91.1–99.4]b,c	95.26 (1,205) [93.9–96.3]c

In each row, values [confidence intervals] followed by the same lowercase letter are not significantly different from each other ($P > 0.05$). Values in parentheses are the number of the adult mosquitoes analyzed. N.S., value for the same mosquito species not significantly different from the control ($P > 0.05$).

[a] *Ma. africana* and *Ma. uniformis* (the few mosquitoes other than these two species and *An. gambiae* s.l. were discarded).

[b] Total number of mosquitoes collected for the entire 20 wk.

[c] 100 × (total no. of mosquitoes in the treated hut/total no. of mosquitoes in the control hut).

[d] 100 × (total no. of mosquitoes in the veranda/total no. of mosquitoes in the hut).

[e] 100 × ((% exophily in the treated hut − % exophily in the control hut)/(100 − % exophily in the control hut)).

[f] 100 × (total no. of mosquitoes blood fed/total no. of mosquitoes).

[g] Blood-feeding inhibition = (1 − (% blood fed in the treated hut/% blood fed in the control hut)) × 100.

[h] Mortality (immediate or delayed): 100 × (total no. of dead mosquitoes/total no. of mosquitoes in the hut).

total number blood fed in the control hut (127 *An. gambiae* and 2,047 *Mansonia* spp.), and B_t the total number blood fed in the treatment huts (four and six *An. gambiae*, 25 and 46 *Mansonia* spp., respectively, with coils and LLIH). The personal protective effect against vector and nuisance mosquitoes was very high for both treatments, ranging from 95.3 (*An. gambiae*, LLIH) to 98.8% (*Mansonia* spp., mosquito coils). With a minimum value of 95% personal protection, this small-scale field trial demonstrated that LLIH provided protection similar to mosquito coils against endophagic mosquitoes. This characteristic is the result of a combination of the repellent and irritant effects of permethrin already demonstrated in many studies (e.g., N′Guessan et al. 2001). Moreover, the high mortality (≈93%, i.e., higher than mosquito coils) indicates a mass killing effect may occur if use of LLIH is widespread. The lasting effectiveness (at least 20 wk) is not surprising because both the hammock and blanket benefit from long-lasting technology proven to be effective for at least 4 yr in field conditions (WHO 2001). Moreover, at this level of efficacy, LLIH can be considered to be more cost-effective and user-friendly than mosquito coils, which need to be replaced nightly after 6–8 h of continuous use. However, mosquito coils are widely distributed in developing countries, and they can be purchased individually, whereas a hammock, even with the long-term savings of a long-lasting treatment, represents a heavy initial investment for most potential users. Moreover, the use of LLIHs is not part of the cultural habits in many countries; hence, it does not necessarily reply to the practices, tastes, or expectations of potential users. However, in other countries, sleeping outdoors in hammocks is widespread. In these situations, further testing of LLIHs for use against exophagic vectors is required. This could be first envisaged in regions where hammocks are widely used such as in jungle areas of South America or South-East Asia. Indeed, Chatterjee (2005) noted that forest workers in Cambodia sleep under nets at night but remain unprotected when they sit outdoors until late evening. A large-scale intervention study using insecticide-treated hammocks is currently being conducted in Vietnam to assess the level of protection from malaria transmission of forest workers that use hammocks at dusk and night.

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