Eave Screening and Push-Pull Tactics to Reduce House Entry by Vectors of Malaria

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Abstract. Long-lasting insecticidal nets and indoor residual spraying have contributed to a decline in malaria over the last decade, but progress is threatened by the development of physiological and behavioral resistance of mosquitoes against insecticides. Acknowledging the need for alternative vector control tools, we quantified the effects of eave screening in combination with a push-pull system based on the simultaneous use of a repellent (push) and attractant-baited traps (pull). Field experiments in western Kenya showed that eave screening, whether used in combination with an attractant-baited trap or not, was highly effective in reducing house entry by malaria mosquitoes. The magnitude of the effect varied for different mosquito species and between two experiments, but the reduction in house entry was always considerable (between 61% and 99%). The use of outdoor, attractant-baited traps alone did not have a significant impact on mosquito house entry but the high number of mosquitoes trapped outdoors indicates that attractant-baited traps could be used for removal trapping, which would enhance outdoor as well as indoor protection against mosquito bites. As eave screening was effective by itself, addition of a repellent was of limited value. Nevertheless, repellents may play a role in reducing outdoor malaria transmission in the peridomestic area.

INTRODUCTION

Malaria remains one of the most deadly infectious diseases and continues to claim hundreds of thousands of lives annually, mostly those of young children in sub-Saharan Africa.^{1,2} The principle prevention strategy is vector control, which largely depends on long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS).¹ Although LLINs and IRS have contributed to a substantial decline in malaria transmission over the last decade,² these intradomestic measures do not target outdoor-feeding mosquitoes, which limits their potential to eliminate malaria completely,^{3,4} particularly in areas where residual malaria transmission occurs outside the home. Moreover, the progress made is threatened by the development of physiological and behavioral resistance of mosquitoes against the insecticidal compounds used.^{5,6} Recent reports confirming the rapid spread of insecticide resistance underline the need for alternative approaches in addition to insecticide-based methods.^{7–12} In this study, we aimed to quantify the effects of eave screening in combination with a push-pull system that interferes with mosquito host-seeking behavior through the simultaneous release of repellent volatiles from the house eaves to “push” mosquitoes away from the peridomestic space and attractive volatiles released from an odor-baited trap to “pull” mosquitoes toward this alternative, pseudohost and remove them from the environment. Push-pull is a term originally adopted in the context of agricultural pest management.^{13,14} A push-pull system manipulates the behavior and/or distribution of the target species by the simultaneous use of repellent and attractive stimuli. The design of an effective

push-pull system directed at malaria vectors is a recent, and ongoing, development.^{15,16}

Throughout the tropics, many traditional houses are constructed with eaves (openings between the top of the wall and the roof), which serve to increase airflow in the houses, but also form the predominant entry point for anopheline mosquitoes.^{17–19} Reducing mosquito house entry by screening eaves and other openings has played a well-documented role in reducing the incidence of malaria in many different countries around the globe.²⁰ Numerous studies show that eave and window screens, or net ceilings, reduce mosquito house entry and in some cases anemia (an indicator of malarial morbidity) in different African countries,^{21–23} and a growing body of evidence supports the concept of improved housing as a means of malaria control.²⁴ However, the practicalities and social acceptance of applying house screening is difficult in many traditional houses that are commonly found in rural areas of sub-Saharan Africa. The many cracks and uneven edges hinder the complete closure of the eave, or other openings, with mesh or netting. The addition of a spatial repellent “push” to the mesh or netting used to screen eaves or other openings could provide a solution for the requirement to completely screen all of the openings.

Key to a functional push-pull system is the controlled, long-lasting, release of repellent and attractive compounds. The use of certain microencapsulation techniques allows for a prolonged and passive release of active volatiles.²⁵ Microcapsules loaded with the desired volatiles can be impregnated into many different kinds of textile fabric to achieve a controlled release of mosquito repellent. The impregnated textile material could then be used to fabricate bed nets or eave screens.^{26,27}

Menger and others¹⁶ showed that a narrow strip of cotton net fabric that was impregnated with microcapsules containing delta-undecalactone (dUDL) and placed in the eave of traditional houses in western Kenya reduced mosquito house entry by 50% or more. The repellency of dUDL against anopheline and other mosquitoes has been previously demonstrated in

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laboratory and semifield settings.^{15,28} Durable textiles could also be used for eave screening, rather than the metal wire mesh, which has been traditionally used. The use of textile for eave screening makes it possible to integrate a repellent barrier with the physical barrier that eave screening provides. It would not be necessary for eave screens impregnated with a long-lasting spatial repellent to close off all holes and cracks in the wall of a house as the impregnated screens would provide a chemical barrier as well.¹⁶ We hypothesized that the combined application of eave screening and push-pull might yield an intervention, which is suitable for use in traditional homes in rural Africa and which is superior over either approach alone.

Attractive synthetic mosquito lures, which have a similar or greater degree of attraction as a human odor, are now available for the monitoring and control of malaria vectors.^{29–31} An ongoing large-scale intervention study explores the potential of mass-trapping malaria vectors to reduce malaria transmission on an island community in western Kenya.³² By actively removing mosquitoes from the peridomestic environment, attractant-baited traps can provide a dual-protective effect: 1) a direct effect by reducing house entry of mosquitoes that would otherwise have entered^{16,33} and 2) an indirect effect by reducing the average mosquito's life span and by depleting mosquito populations through daily removal trapping.^{34,35}

A pilot experiment in which a human-occupied house in a semifield setup was fully screened (eaves and other openings) and equipped with an attractant-baited trap hanging outside the house indicated that this approach could dramatically reduce house entry of malaria vectors and enhance attractant-baited trap catches. Additionally, this pilot showed that the effect of the full screening was superior to the passive release of a repellent from the eave without screening (data not shown).

Here, we present the results of two field experiments in which we studied the effects of eave screening using various untreated or repellent-impregnated materials, alone or in combination with an attractant-baited trap, on house entry and outdoor trap catches of malaria vectors.

The first experiment addresses the effects of traditional eave screening with wire mesh as well as the deployment of an attractant-baited trap in a malaria endemic village in western Kenya. The second experiment, conducted several months later in the same village, investigates the effects of repellent-impregnated versus untreated cotton net fabric, both in the presence and in the absence of outdoor, attractant-baited traps.

MATERIALS AND METHODS

Study site. Both experiments were carried out in Kigoche village in Kisumu County, Kenya (00°08'19" S 34°55'50" E, altitude: 1,160 m) (as previously described^{16,31}). Kigoche village is located within the Ahero rice irrigation scheme and typically experiences two rainy seasons: long rains between March and August and short rains between October and November, with an average annual rainfall of between 1,000 and 1,800 mm. The mean relative humidity is 65% and the temperature ranges from 17°C to 32°C. These climatic conditions and the rice irrigation scheme mean that mosquito breeding sites are found in Kigoche throughout the year.

Traditional, mud-walled houses with a minimal distance of 25 m between them were selected for the experiments. Other (unselected) houses were present around and in between the study houses.

Measuring mosquito house entry. Eight houses were selected for field experiment I and 12 houses were selected for field experiment II (as described in the section Experimental design below). Male volunteers between 18 and 28 years of age were recruited to sleep in the houses, one person per house, to attract mosquitoes. Volunteers rotated in a strict order between the houses on a nightly basis to minimize the influence of differences in individual attractiveness. During both experiments mosquito entry was measured using an unlit Centers for Disease Control and Prevention (CDC) light trap that was suspended at the foot end of the bed with the rain cover hanging approximately 15 cm above the mattress.^{36,37} Each experimental night started at 19:30 hours and finished at 06:30 hours the following morning.

Experimental design. *Field experiment I.* A baseline experiment took place during eight nights in all eight houses (i.e., one full rotation of the eight human volunteers) while no eave screening or traps were present. The four interventions tested during the subsequent intervention phase were (I) the control treatment, that is, no eave screening or attractant-baited trap, (II) eave screening with wire mesh only, (III) an attractant-baited trap only, and (IV) eave screening with wire mesh and an attractant-baited trap combined. Each intervention was randomly assigned to two houses. Interventions were not rotated among houses to allow eave screens to be installed for the full duration of the study. In all treatments, the volunteers slept underneath untreated bed nets.

The whole experiment took place over a period of 33 consecutive nights in May and June 2014; eight nights for the baseline phase, one to install the eave screens, and 24 nights during the intervention phase (three complete rotations of the eight human volunteers). Indoor CDC light traps and outdoor, attractant-baited traps (see below) were taken to the field laboratory after each experimental night, where mosquitoes were frozen and identified (see section Species identification below).

Experiment I materials. To screen the eaves, wire mesh (hole size of 2-mm diameter) was cut into strips of 50 cm width, sufficiently wide to cover eaves, which had widths ranging from ca. 15 to 30 cm. Wire mesh was applied from the outside of the houses. It was first fixed to the lower part of the eave using staples or nails and then stretched upward to the corrugated iron sheet roof and clamped around the wooden beams supporting the roof (Supplemental Figure 1). Gaps between the wooden beams and the wire mesh were filled with cotton wool. However, due to the corrugated structure of the roof, it was not possible to close the eaves completely. To help stretch the wire mesh and hold it flush against the roof, wooden sticks were placed into the eave at regular intervals.

The attractant-baited trap chosen for this experiment was the Suna trap (Biogents AG, Regensburg, Germany), a novel type of counterflow trap that was recently developed as a tool for mosquito monitoring and control.³³ It was baited with an attractive five-compound odor blend released from nylon strips,^{28,30} augmented with carbon dioxide (CO₂) produced by the fermentation of molasses.³⁸ Fresh odor baits were provided at the start of the experiment and left in place

throughout the entire trial as previous studies have shown that the strips remain attractive for at least 52 nights after impregnation.^{39,40} CO₂ was provided nightly, at the start of each experimental night. The Suna trap was hung outside the house, next to the door, suspended from the overhanging roof with a nylon line, with the air inlet positioned at 30 cm above ground level.³³

Field experiment II. In the second field experiment, conducted in October and November 2014, cotton net fabric was used for eave screening instead of wire mesh. The treatments tested were the following: (I) control, that is, no eave screening; (II) eave screening with untreated net fabric; (III) eave screening with net fabric impregnated with microencapsulated dUDL repellent to “push” mosquitoes away from the peridomestic space (see below for microencapsulation method); and (IV) eave screening with net fabric that was treated each day with a spray-on para-menthane-3,8-diol (PMD) based repellent (see below), which also acts as a “push.” During the intervention phase, attractant-baited traps were placed outdoors at every house, including the control houses, every second night (see below). Attractant-baited traps acted as a pseudohost, a “pull,” to capture mosquitoes, which were “pushed” from houses by either of the repellents.

Twelve houses were included in this experiment, seven of which had also been used in field experiment I, plus five other houses. The baseline experiment took place over 12 consecutive nights in all houses to allow one full rotation of all human volunteers, while none of the intervention measures were applied. Based on the mean CDC trap catch during the baseline phase, houses were classified as high, medium, or low mosquito-entry houses, with four houses in each group. Within each group, one house was randomly assigned to one of the treatments (thus resulting in three houses per treatment).

The experiment consisted of 12 nights of trapping to collect baseline data, followed by 24 nights of trapping during which the interventions/treatments were installed, with attractant-baited traps being deployed every other night. Nightly indoor CDC trap catches were used as a proxy for mosquito house entry. Outdoor, attractant-baited trap catches were counted in the morning after the traps had been operated overnight.

Experiment II materials. The fabric that was used for each treatment was a 100% sheer cotton gauze fabric that was specially designed for this purpose (Leno weave structure, 65 g/m², provided by Utebel NV, Ronse, Belgium). It was fixed using a staple gun, and rather than applying it to cover only the eave, it was stapled to the wooden beam that forms the top of the wall and then stretched outward and fixed on the outermost beam, which supports the roof (Supplemental Figure 2). This method had the advantage that there were two solid beams to stretch the fabric between, which allowed a fast and efficient installation and enabled the eave to be closed off effectively without working around the radial beams and spaces created by the corrugation of the roof.

Microcapsules containing dUDL were produced and applied as described previously.¹⁶ The microcapsules consisted of 31% wt. dUDL and were applied on the substrate by padding, obtaining a wet pickup of 60%. The resulting fabric contained 3 g of dry microcapsules/m².

For the PMD treatment, a commercially available repellent that contained 192 g/L Citriodiol™ (approximately 64%

PMD) was sprayed on the fabric inside the eave, right before the start of each experimental night, applying 0.14 g (1 puff) per running meter.

Mosquito Magnet X (MM-X) traps (American Biophysics, North Kingstown, RI)^{41,42} were used as outdoor, attractant-baited traps during experiment II, as they were found to better preserve the trapped mosquitoes than Suna traps. They were set up identically to the Suna traps used in the previous experiment, with the exception that the air outlet was positioned at 15 cm above ground level as this has previously been shown to be the optimal positioning for this type of trap.⁴³

Species identification (both experiments). Trapped mosquitoes were killed in a freezer and morphologically identified.⁴⁴ Culicine mosquitoes were identified to genus level and anophelines were identified as *Anopheles funestus* sensu lato (s.l.), *Anopheles gambiae* s.l., and other *Anopheles* spp. *Anopheles funestus* s.l. and *An. gambiae* s.l. mosquitoes were placed into 2-mL Eppendorf tubes with silica gel and a piece of cotton wool to preserve them for subsequent species identification. DNA was extracted from individual specimens and a 153- to 780-base pair region of the intergenic transcribed spacer region 2 (ITS2) of the ribosomal DNA (rDNA) gene was polymerase chain reaction (PCR) amplified using previously described primer sets.^{45,46} All amplicons were sequenced and subjected to phylogenetic analysis to differentiate members of the *An. gambiae* and *An. funestus* complexes. The abdominal status of female mosquitoes was categorized as unfed, blood-fed, or gravid.

Statistical analysis. Data from the baseline experiments allowed for the correction of initial differences in the mosquito entry rate between houses using a difference-in-differences method.^{16,47} The difference in mosquito house entry between the baseline and the intervention phase was determined for each house by calculating the percentage difference in catch size of each mosquito species compared with the baseline value: difference (%) = (baseline catch size – Intervention catch size)/baseline catch size × 100. Further analyses were performed using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY). For field experiment I, the difference in mosquito house entry of all three intervention treatments was compared with the difference observed in the control houses during the time when the intervention was applied in other houses. Mann–Whitney *U* (MWU) tests corrected for multiple comparisons with the Benjamini–Hochberg procedure (false discovery rate = 0.05) used to test for statistical significance of these differences. For field experiment II, all treatments (including the control treatment) were compared with each other using Scheffé’s post hoc tests. This analysis was performed separately for when outdoor, attractant-baited traps were absent and for when they were present. For each separate treatment, house entry reduction with or without outdoor traps was compared using an MWU test. Finally, outdoor trap catches in both experiments were compared between houses where eave screening was present and houses where it was absent using MWU tests corrected for multiple comparisons using the Benjamini–Hochberg procedure.

Ethical statement. These experiments were part of a study that was approved by the ethical review committee of the Kenya Medical Research Institute (KEMRI/RES/7/3/1). House owners and volunteers were informed about the purpose and procedures of the experiments and consented by

signing, after having read and understood the consent form approved by the ethics committee of KEMRI. During the study there was daily communication with the volunteers, who were screened for malaria on a weekly basis and had continuous access to artemisinin combination therapy in case of uncomplicated malaria infection.

RESULTS

Field experiment I. A total of 7,305 mosquitoes were trapped using CDC light traps inside the houses over the entire experiment (96% female and 4% male). Anophelines comprised 62% (4,496) of the total catch and the remaining 38% (2,809) were culicines. Among the anophelines, 95% were *An. funestus* s.l., 5% *An. gambiae* s.l., and < 0.1% other anophelines. The culicine population comprised 99.6% *Culex* spp. and 0.4% *Mansonia* spp.

In the outdoor Suna traps, a total of 5,180 mosquitoes were caught (97% female and 3% male). Of these, 39% (1,999) were anophelines and 61% (3,181) were culicines. Among anophelines, 87% were *An. funestus* s.l., 4% *An. gambiae* s.l., and 9% other anophelines (including *Anopheles coustani*, *Anopheles ziemanni*, and other unidentified species). Among collected culicines, 76% were *Culex* spp. and 24% were *Mansonia* spp.

A subsample of 152 *An. funestus* s.l. females collected from inside and outside houses was analyzed for subspecies composition by PCR. Of the 142 samples that were successfully amplified, all were *An. funestus* s.s. Of 158 *An. gambiae* s.l. females that were analyzed by PCR, 156 were successfully amplified and all were *Anopheles arabiensis*. Further results of intervention effects are reported for *An. funestus*, *An. arabiensis*, and *Culex* spp. only. For these three groups, the abdominal status and sex of the trapped mosquitoes is summarized in Table 1.

Mean indoor CDC light trap catches for each house can be found in Supplemental Table 1. Figure 1 shows the differences in house entry between the control and each of the treatments for *An. funestus*, *An. arabiensis*, and *Culex* mosquitoes. All reported values are percentage differences compared with the baseline mean.

House entry of *An. funestus* in the control houses was 4% higher during the intervention period than during baseline. When attractant-baited Suna traps were used, mean house entry was reduced by 18%. Eave screening alone reduced house entry by 92% and the combination of a Suna trap +

eave screening resulted in a mean house entry reduction of 90%. The reductions in house entry in houses that were screened, whether a Suna trap was present, were significantly greater than the difference observed in the control treatment (MWU tests, $P < 0.001$).

For *An. arabiensis*, indoor trap catches during the baseline phase were low (a mean of 2.1 mosquitoes per house per night), and the calculation of percentage differences in house entry led to more extreme values and greater variation in estimated means than for the other species. In the control houses, mosquito entry was 114% higher during the intervention period compared with the baseline. With a Suna trap in place, house entry was 13% lower than during baseline. Eave screening, either in combination with a Suna trap or alone, reduced house entry by 99%. The effect of both eave screening treatments was statistically significant (MWU tests, $P < 0.001$).

For *Culex* spp., house entry during the intervention phase was 31% lower in the control houses, compared with the baseline mean. In houses with a Suna trap placed outside, there was a decrease in house entry of 44%. Eave screening alone reduced house entry by 92%, and for the combination of a Suna trap + eave screening a reduction of 87% was measured. The reductions by the two treatments that included eave screening were significantly greater than the decrease observed in the control treatment during the same time period (MWU tests, $P < 0.001$).

Suna trap catch sizes were compared between the treatment with a Suna trap only versus a Suna trap with eave screening (Table 2). For *An. funestus*, Suna trap catches were 42% higher when the trap was deployed in addition to eave screening, compared with when the trap was installed alone (MWU, $P = 0.038$). For *An. arabiensis*, however, Suna trap catches were 36% lower at houses where the eaves were screened (MWU, $P = 0.040$), although mean trap catches were only 1.1 and 0.7 mosquitoes per house per night, respectively. Suna trap catches of *Culex* spp. were 30% lower when eaves were screened, but this difference was not significant (MWU, $P = 0.418$).

Field experiment II. During the second field experiment, a total of 4,137 mosquitoes were trapped inside the houses (96% female and 4% male). Of these, 79% (3,266) were anophelines and the remaining 21% (871) were culicines. Among anophelines 75% were *An. funestus* s.l. and 25% *An. gambiae* s.l. The culicine population was composed of 97% *Culex* spp. and 3% *Mansonia* spp.

TABLE 1

Abdominal status and sex of indoor CDC and outdoor Suna trap catches for *Anopheles funestus*, *Anopheles arabiensis*, and *Culex* spp. during experiment I

Experiment 1					
Species	Female			Male (%)	Total
	Unfed (%)	Blood-fed (%)	Gravid (%)		
Indoor CDC trap catches					
<i>An. funestus</i>	4,037 (94.5)	38 (0.9)	42 (1.0)	156 (3.7)	4,273
<i>An. arabiensis</i>	192 (87.7)	10 (4.6)	7 (3.2)	10 (4.6)	219
<i>Culex</i> spp.	2,592 (92.7)	20 (0.7)	23 (0.8)	162 (5.8)	2,797
Outdoor Suna trap catches					
<i>An. funestus</i>	1,641 (94.7)	9 (0.5)	6 (0.3)	76 (4.4)	1,732
<i>An. arabiensis</i>	82 (95.3)	3 (3.5)	0 (0.0)	1 (1.2)	86
<i>Culex</i> spp.	2,312 (95.8)	6 (0.2)	11 (0.5)	85 (3.5)	2,414

CDC = Centers for Disease Control and Prevention. For indoor CDC trap catches, numbers are the total of 32 trapping nights in eight houses during the baseline and the intervention phase. For outdoor Suna trap catches, numbers are the total of 24 trapping nights for four houses during the intervention phase.

FIGURE 1. Differences in house entry during the intervention phase compared with baseline, experiment I. Bars show the mean $\pm$ standard error of mean, $N = 48$ trap nights for all groups. In houses that received a treatment that included eave screening, there was a significant decrease in mosquito entry compared with the control houses, $*P < 0.001$ and ns = not significant (Mann-Whitney U tests corrected for multiple comparisons with the Benjamini-Hochberg procedure with a false discovery rate of 0.05).

TABLE 2

Mean number of mosquitoes caught outdoors per trap per night $\pm$ SEM, with Suna trap only and Suna trap with eave screening during experiment I

Species	Suna only	Suna + eave screening
<i>Anopheles funestus</i>	14.9 $\pm$ 1.5	21.2 $\pm$ 2.1*
<i>Anopheles arabiensis</i>	1.1 $\pm$ 0.2	0.7 $\pm$ 0.2*
<i>Culex</i> spp.	29.5 $\pm$ 4.2	20.8 $\pm$ 2.3

SEM = standard error of mean. Values are based on 24 trapping nights with two houses per treatment ($N = 48$).

* Indicate a significant difference at $\alpha = 0.05$ between treatments (Mann-Whitney U tests).

In the outdoor MM-X traps, a total of 7,471 mosquitoes were caught (88% female and 12% male). Of these, 35% (2,620) were anophelines and 65% (4,851) were culicines. Among anophelines, 38% were *An. funestus* s.l., 48% were *An. gambiae* s.l., and 13% were other anopheline species (including *An. coustani*, *An. ziemanni*, and other unidentified spp.). Among culicines, 58% were *Culex* spp. and 42% *Mansonia* spp.

Anopheles funestus complex differentiation was performed on a subsample of 72 outdoor-trapped and 72 indoor-trapped *An. funestus* s.l. individuals by PCR amplification, sequencing, and phylogenetic analysis of a region of the rDNA ITS2. Of these trapped *Anopheles*, 142 were *An. funestus* s.s., while two were *An. arabiensis* that had been incorrectly morphologically identified (Supplemental Figure 3A). *Anopheles gambiae* complex differentiation was performed on a subsample of 70 outdoor-trapped and 72 indoor-trapped *An. gambiae* s.l. individuals by PCR amplification, sequencing, and phylogenetic analysis of a region of ITS2. One hundred and thirty-eight individuals yielded PCR amplicons, all of which were *An. arabiensis* (Supplemental Figure 3B). Further results are reported for *An. funestus*, *An. arabiensis*, and *Culex* spp. For these three groups, the abdominal status and sex of the trap catches is given in Table 3.

The mean indoor CDC light trap catches per house during the baseline and the intervention phase are reported in Supplemental Table 2. Figure 2 shows the differences in house entry between the intervention phase and the baseline for all treatments (including the control treatment). House entry of *An. funestus* in the control houses was 11% lower during the intervention phase than during the baseline phase. Eave screening with cotton net fabric reduced house entry of *An. funestus* by 61%. Eave screening with fabric that was impregnated with microencapsulated dUDL reduced house entry by 63%. Eave screening with fabric that was sprayed with PMD before each experimental night reduced house entry by 81%. All eave screening treatments significantly reduced *An. funestus* house entry (Scheffé's post hoc test, Figure 2).

With an MM-X trap in place, house entry of *An. funestus* in the unscreened control houses decreased by 22% compared with the baseline mean. An MM-X trap in combination with eave screening with cotton net fabric reduced *An. funestus* entry by 62%. The combination with fabric that was impregnated with microencapsulated dUDL reduced house entry by 35%. An MM-X trap in combination with PMD-treated fabric reduced house entry by 80%. When comparing all treatments that included an outdoor MM-X trap, only the combination with fabric that was sprayed with PMD resulted in a significantly greater house entry reduction compared with unscreened houses with an MM-X trap. However, there was no significant difference with the effects of

TABLE 3
Abdominal status and sex of trap catches for *Anopheles funestus*, *Anopheles arabiensis*, and *Culex* spp. during experiment II

Species	Female			Male (%)	Total
	Unfed (%)	Blood-fed (%)	Gravid (%)		
Indoor CDC trap catches					
<i>An. funestus</i>	2,311 (94.8)	48 (2.0)	15 (0.6)	63 (2.6)	2,437
<i>An. arabiensis</i>	732 (88.3)	29 (3.5)	18 (2.2)	50 (6.0)	829
<i>Culex</i> spp.	744 (88.4)	40 (4.8)	2 (0.2)	56 (6.7)	842
Outdoor MM-X trap catches					
<i>An. funestus</i>	930 (92.4)	5 (0.5)	1 (0.1)	70 (7.0)	1,006
<i>An. arabiensis</i>	1,031 (81.8)	46 (3.6)	19 (1.5)	165 (13.1)	1,261
<i>Culex</i> spp.	2,391 (84.8)	239 (8.5)	1 (< 0.1)	190 (6.7)	2,821

CDC = Centers for Disease Control and Prevention; MM-X = Mosquito Magnet X. For indoor CDC trap catches, numbers are the total of 36 trapping nights in 12 houses, during the baseline and the intervention phase. For outdoor MM-X trap catches, numbers are the total of 12 trapping nights for 12 houses during the intervention phase.

the other eave screening treatments (Scheffé's post hoc test, Figure 2). The effect of adding an MM-X trap to any of the eave screening treatments or the control was not significant (MWU tests, $P > 0.05$ for all comparisons, not shown).

House entry of *An. arabiensis* was 11% lower in the control houses during the intervention phase. Eave screening led to a reduction of 72%. Eave screening with dUDL reduced house entry by 83%. Eave screening with PMD-treated fabric reduced house entry by 89%. All eave screening treatments significantly reduced house entry of *An. arabiensis* (Scheffé's post hoc test, Figure 2).

With an MM-X trap in place, house entry of *An. arabiensis* into unscreened control houses decreased by 30%. An MM-X trap in combination with eave screening led to a house entry reduction of 65%. The combination of an MM-X trap with dUDL-impregnated fabric reduced house entry by 55%. With PMD-treated fabric and an MM-X trap, the reduction was 80%. Only the combination with PMD-treated fabric reduced house entry of *An. arabiensis* significantly when compared with the control houses with an outdoor MM-X trap. However, the difference with the effects of the other eave screening treatments was not significant (Scheffé's post hoc test, Figure 2). There was no significant effect of the presence or absence of an MM-X trap on house entry reduction for any of the treatments or the control (MWU tests, $P > 0.05$ for all comparisons, not shown).

House entry of *Culex* spp. was 36% lower in the control houses during the intervention phase compared with the baseline mean. With eave screening, house entry of *Culex* spp. was 54% lower. When dUDL-impregnated fabric was used, the reduction was 35%. Eave screening with PMD-treated fabric reduced house entry by 84%. None of these reductions was significant compared with the difference observed in the control houses, notwithstanding the relatively large effect size of the treatment with PMD-treated fabric (Scheffé's post hoc test, Figure 2).

In houses with an MM-X trap placed outside, the decrease in house entry of *Culex* spp. was 43%. For houses that received both eave screening and an MM-X trap, a reduction of 74% was observed. When dUDL-impregnated fabric was used in combination with an MM-X trap, the reduction was 59%. An MM-X trap in combination with PMD-treated fabric reduced house entry by 83%. The combination of an MM-X trap and fabric that was sprayed with PMD reduced house entry significantly more than the MM-X trap used alone at unscreened houses. However, there was no significant difference with the effects of the other eave screening

treatments (Scheffé's post hoc test, Figure 2). For all treatments, the degree of house entry reduction with or without an MM-X trap was similar (MWU tests, $P > 0.05$ for all comparisons, not shown).

MM-X trap catches were compared for the four treatments that included the placement of an MM-X trap. For all species MM-X trap catches were higher when the treatment included eave screening compared with when the trap was used at unscreened control houses (see Table 4 for statistical significance). The increase in mosquito catches ranged from 36% up to 110%.

DISCUSSION

Both of the field experiments showed that eave screening, used alone or in combination with an attractant-baited trap, was very effective in reducing house entry by anopheline mosquitoes. The magnitude of the effect varied between the different anopheline species and between the two experiments, but the effect was always considerable (between 61% and 99%) and statistically significant.

For *Culex* spp. house entry was reduced whenever eave screens were installed, although the reductions measured in field experiment II were substantially smaller than in experiment I and were not statistically significant. Partly, this may be explained by *Culex* mosquitoes being less affected by eave screening than *Anopheles* mosquitoes¹⁹ due to the preference of culicines for entering houses through doors and windows. However, the limited effect size and lack of statistical significance in experiment II can also be explained by a much lower overall house entry of *Culex* spp. during the intervention phase compared with baseline. Most likely these observations were caused by natural population fluctuations occurring during periods with heavy showers, as the second field experiment took place during the start of the short rainy season in 2014. *Culex* spp. are known to be very sensitive to rains, in terms of population dynamics as well as larval survival rates.^{48,49} Although *Culex* spp. are not vectors of malaria, they are able to transmit lymphatic filariasis and they are nuisance biters, which makes their control important both from a medical viewpoint and for the social acceptability of the intervention.^{50–52} Conducting both experiments simultaneously would have allowed for comparison of wire mesh screening with fabric screening under the same conditions. Unfortunately logistical limitations meant that a simultaneous comparison was not possible during this study, but this could be addressed in future experiments.

FIGURE 2. Differences in house entry during the intervention phase compared with baseline, experiment II. Bars show the mean $\pm$ standard error of mean, $N = 36$ trap nights for all groups. When houses received eave screening, there was a significant reduction in the house entry of *Anopheles funestus* and *Anopheles arabiensis* compared with unscreened control houses; reductions in the house entry of *Culex* spp. were not significant (Scheffé's post hoc test, lowercase letters, bars not sharing the same letter are significantly different at $\alpha = 0.05$). When an outdoor Mosquito Magnet X (MM-X) trap was present, the combination with eave screening was always more effective than the outdoor trap alone, although this effect was only significant when eave screens were treated with para-menthane-3,8-diol (PMD). (Scheffé's post hoc test, uppercase letters, bars not sharing the same letter are significantly different at $\alpha = 0.05$). For all treatments (including the control), the degree of house entry reduction was not significantly affected by the presence or absence of an MM-X trap (Mann-Whitney U tests, $P > 0.05$ for all comparisons).

Other studies that looked into the use of physical barriers to reduce mosquito house entry also report consistent though variable reductions. Lindsay and others²³ found that installing ceilings made of different materials reduced mosquito house entry by 59–80%. Kirby and others²² reported that full-house screening or the installation of screened ceilings reduced house entry of *An. gambiae* s.l. by around 50% and, moreover, was associated with significantly reduced anemia in children. Several later studies confirmed that screening the house entry points of mosquitoes can significantly reduce the entry of malaria vectors and other mosquitoes, although the effect size differs per species and according to the method of screening used.^{21,51}

The method of screening that was used in field experiment I (wire mesh, see section Materials and Methods for details on the application technique) yielded house entry reductions between 87% and 99%, whereas the method that was used in field experiment II (cotton net fabric, see the section "Materials and Methods") resulted in reductions of between 61% and 74%. In part, this difference may be explained by the application of cotton wool to close gaps between the house's structure and the wire mesh in field experiment I. However, it was also observed that the net fabric would tear at the places where it was fixed, if it was not applied as a double layer. It was also noted that the net fabric would lose its elasticity after a number of days, which resulted in the formation of narrow openings at the top of the wall and at the wooden beams. The performance of the net fabric could probably be improved by using doubled up fabric as a standard and by stapling it to the wood at shorter intervals, though this thicker layer of fabric may have a negative impact on air circulation in the house. Quantification of the rate of deterioration of the net fabric was not made during this study, but development of longer-lasting fabric is an issue that could be addressed during future product development. Overall, when only eave screening, without the application of repellents is the aim, the wire mesh would probably be the superior material based on its durability and robustness. A similar conclusion was drawn by Kirby and others,⁵³ who recommended future research on house screening to focus on materials with a high robustness.

When the net fabric eave screen was impregnated with microencapsulated dUDL, no significant changes in mosquito house entry were observed. Menger and others¹⁶ observed that the application of a narrow strip of dUDL-impregnated fabric reduced house entry of anopheline mosquitoes by around 50%. The reason that dUDL did not improve the effect of eave screening may be that eave screening itself was already so effective that most of the mosquitoes trapped inside screened houses did not enter via the eave or surrounding gaps and cracks, but through other openings, which were outside the range of effective dUDL release (e.g., through the door if it was left open during the early evening or not properly closed during nighttime and through gaps between the sheets of corrugated iron that made up the roofs of the selected houses). Assuming this was the case we can deduce that although dUDL impregnated fabric has a spatial effect as shown in Menger and others,¹⁶ this repellent effect is not large enough to completely prevent house entry when the fabric is only applied inside the eave. Based on the width of the eaves (approximately 30 cm) and the size of the houses, we can then roughly estimate the spatial effect of the dUDL fabric to be

TABLE 4

Mean number of mosquitoes caught outdoors per trap per night $\pm$ SEM in MM-X traps placed outdoors next to control houses and houses with various types of eave screening during experiment II

Species	Control	Eave screening	Eave screening dUDL	Eave screening PMD
<i>Anopheles funestus</i>	5.0 $\pm$ 0.7	8.1 $\pm$ 1.5	8.2 $\pm$ 1.2	6.8 $\pm$ 1.1
<i>Anopheles arabiensis</i>	5.9 $\pm$ 0.9	8.2 $\pm$ 1.1	12.4 $\pm$ 1.9*	8.6 $\pm$ 1.2
<i>Culex</i> spp.	14.5 $\pm$ 3.7	20.9 $\pm$ 3.3*	22.9 $\pm$ 3.2*	20.0 $\pm$ 3.4*

dUDL = delta-undecalactone; MM-X = Mosquito Magnet X; PMD = para-menthane-3,8-diol; SEM = standard error of mean. Values are based on 12 trapping nights, with three replicates per treatment per night ($N = 36$).

*Indicate a significant difference compared with the control group (Mann-Whitney U tests, after Benjamini-Hochberg procedure with a false discovery rate of 0.05).

between 20 and 100 cm; a more precise experiment would be needed to confirm this estimation.

Spraying the cotton eave screen with the repellent PMD was associated with a greater house entry reduction by all mosquito species analyzed. Although this effect was consistent it was too small to be significant, at least in part because there was little room for improvement as the untreated cotton was already quite an effective mechanical barrier by itself. As PMD was sprayed on to the fabric at the onset of each experimental night, the concentration of volatile PMD in the surrounding air must have been relatively high during the first hours of the night. Indeed, volunteers reported that the smell of PMD was clearly present around the house after application, which was not the case for dUDL.

While the use of spatial repellents did not lead to additional reductions in house entry beyond those already achieved through eave screening, the use of spatial repellents may still confer advantages which were not measureable in this setup. The application of spatial repellents (in this case to the eave screening) is expected to reduce encounters between humans and mosquitoes, thus reducing mosquito bites in the peridomestic area in daytime and evening hours. The application of an effective spatial repellent is also expected to increase the duration of host searching, thus prolonging the exposure of mosquitoes to outdoor conditions, which may in turn shorten the life span of a mosquito, thus reducing vectorial capacity.⁵⁴ It has also been shown that exposure to some spatial repellents has a long-lasting detrimental effect on host-seeking behavior in *Aedes albopictus*.⁵⁵

The addition of an outdoor, attractant-baited trap to unscreened control houses was associated with reductions in house entry for all mosquito species in both field experiments (though not statistically significant). When added to houses with the various types of eave screening, the effect of an outdoor trap on mosquito house entry was variable. Previous studies reported effects of outdoor, attractant-baited traps on house entry of mosquitoes ranging from absent⁴³ to reductions of around 40%.^{16,33} In several semifield studies, reductions of between 33% and 82% were observed, although this may partly be explained by the limited number of mosquitoes released in such setups.^{15,33} The main aim of installing attractant-baited traps, however, would be to deplete mosquito populations through daily removal trapping.^{34,35,56} In both field experiments, outdoor traps caught considerable numbers of malaria vectors and other mosquitoes. In field experiment II, MM-X traps hung outside houses to which a type of eave screening had been applied, trapped consistently higher numbers of mosquitoes of all species than traps outside unscreened houses. The results of field experiment I were more variable. When taken together, higher outdoor trap catches were associated with eave screen-

ing in 10 of 12 cases, with increases up to 110%. This is noteworthy, because in studies in which only a repellent barrier was used to reduce mosquito house entry, no increases in outdoor trap catches were observed.^{15,16} Similar to our findings, a recent study in Belize indicated that outdoor, attractant-baited trap catches were higher for *Anopheles vestitipennis* when transfluthrin-impregnated strips were applied inside experimental houses compared with traps not used in combination with a repellent.⁵⁷

Compared with CDC trap catches inside the houses, both types of outdoor traps caught relatively more *Culex* spp. and fewer *An. funestus*, while catches of *An. arabiensis* were relatively similar both indoors and outdoors. In experiment I, the proportion of blood-fed and gravid mosquitoes trapped outdoors was lower than indoors for all species. In experiment II, the same was true for gravid mosquitoes of all species and blood-fed *An. funestus*, while relatively more *An. arabiensis* and *Culex* spp. were trapped in outdoor, compared with indoor traps. The species composition of indoor trap catches in field experiments I and II also differed. While the fraction of *An. funestus* remained constant, the proportion of *An. arabiensis* was much higher in field experiment II compared with experiment I (20% instead of 3%, respectively), whereas the proportion of *Culex* mosquitoes in experiment II was lower (20% instead of 38%). An explanation may be found in the availability of more temporal breeding sites during experiment II, which took place during the short rainy season, as these are easily colonized by members of the *An. gambiae* complex.⁵⁸

This study shows that, to reduce house entry of malaria and other mosquitoes, eave screening was very effective by itself and the addition of a repellent (dUDL or PMD) was of limited value. As eave screening does not kill mosquitoes, it would be advisable to combine it with an attractant-baited trap for population reduction. Reduction in the mosquito population through mass trapping would offer the additional advantage of protecting people living in unscreened houses or people who are outdoors. Eave screening was found to increase outdoor trap catches, an effect which has not been observed with the use of repellent barriers. A currently ongoing study should provide valuable insights into the feasibility and efficacy of the mass deployment of attractant-baited traps as a tool to reduce malaria transmission.³²

The combination of eave screening and attractant-baited traps could be complementary to insecticide-based vector control tools such as LLINs and IRS. In combination with eave screening, the efficacy of the trap will be enhanced by the presence of the eave screen. With robust eave screens and traps that can operate independently for prolonged periods, the system would be user-friendly and practical for real-world implementation. For eave screening, long-lasting

materials should be used, such as wire mesh. For the traps, long-lasting formulations of odor blends with a high attractiveness already exist.^{39,40} Remaining issues are cost versus effectiveness and the continuous supply of CO₂ and electricity on a large scale, although recent advances in the use and storage of solar energy may resolve the latter in the near future.³²

The possible benefit of impregnating the eave screening material with a repellent would be small and probably not outweigh the extra costs. However, this does not imply that repellents may not play a role in the control of malaria mosquitoes. On the contrary, repellents may still play a key role in reducing outdoor transmission in the peridomestic area. A concern regarding the use of topical repellents is the diversion of mosquitoes from repellent users to unprotected individuals.⁵⁹ However, in an environment in which many traps are deployed, mosquitoes may be diverted to the traps instead. When, in addition, houses are screened, rendering the occupants inaccessible to endophagic mosquitoes, this effect would presumably be enhanced.

Both PMD and dUDL remain interesting candidates for future usage in this context. PMD is derived from the essential oil of *Corymbia citriodora* and is marketed as a natural alternative to DEET. PMD-based repellents have been shown to have an efficacy similar to that of DEET against mosquitoes of several genera, including vectors of human disease, as described by Carroll and Loye.⁶⁰ dUDL was first identified in a structure activity study on olfactory receptor proteins of *An. gambiae* s.s.⁶¹ and was later shown to be a good spatial repellent against anopheline and other mosquitoes in laboratory and (semi-)field settings.^{15,28} It is a natural product that is also found in food sources and has an odor, which is generally described as pleasant.^{62,63} Microencapsulated dUDL or other repellents may be used to impregnate garments or could be added to soaps and shampoos. Repellents with a spatial effect may also provide a degree of protection to outdoor spaces such as cooking areas, which are otherwise hard to protect.

Especially in areas where insecticide resistance is widespread, the introduction of eave screening, alone or in combination with attractant-baited traps, could provide an important additional strategy to be used alongside currently used vector control methods. Where a significant proportion of malaria transmission occurs outdoors, the addition of topical and/or spatial repellents may contribute to enhance protection against mosquito biting. The efficacy of such integrated approaches to reduce malaria transmission should be determined in long-term field experiments, preferably in different ecosystems.

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