

1 **Insecticide-treated combinations of window screens and eave baffles may help control**
2 **physiologically and behaviorally resistant malaria vector mosquitoes**

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17

18 **Abstract**

19 Netting window screens and eave baffles (WSEBs), allowing mosquitoes to enter but not exit
20 from houses, were assessed as an alternative to indoor residual spraying (IRS) for malaria vector
21 control. WSEBs treated with water, the pyrethroid lambda-cyhalothrin (LC), or the
22 organophosphate pirimiphos-methyl (PM), with and without a binding agent (BA) for increasing
23 insecticide persistence on netting, were compared with IRS in experimental huts. Compared with
24 IRS using the same insecticide, WSEBs killed similar proportions of *Anopheles funestus* which
25 were resistant to pyrethroids, carbamates and organochlorines, and greater proportions of
26 pyrethroid-resistant, early-exiting *An. arabiensis*. WSEBs with PM killed greater proportions of
27 both vectors than with LC or LC plus PM, and were equally efficacious when combined with
28 BA. WSEBs required far less insecticide than IRS and BAs may enhance durability. WSEBs
29 may enable affordable deployment of insecticide combinations to mitigate against physiological
30 insecticide resistance, and improve impact upon behaviorally-resistant, early-exiting vectors.

31

32 **Summary**

33 Here we show how insecticide-treated netting window screens and eave baffles may be an
34 efficacious alternative to indoor residual spraying for malaria vector control, to reduce
35 insecticide consumption and enable affordable deployment of insecticide cocktails against
36 physiologically and behaviorally resistant mosquitoes.

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39 **Running title**

40 Insecticidal window screens and eave baffles

41

42 **Keywords**

43 Malaria, *Plasmodium*, vector control, indoor residual spraying, insecticide resistance, residual
44 transmission, behavior, mosquito, *Anopheles*

45

46 **Background**

47 Vector control with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS)
48 interventions account for 78% of the 663 million malaria cases, and most of the four million
49 deaths, averted globally over recent years (1, 2). LLINs and IRS can dramatically reduce malaria
50 transmission by killing sufficient numbers of vector mosquitoes when they attack sleeping
51 humans and/or rest indoors (3-5). However, as these approaches have been scaled up,
52 *physiological* resistance to their insecticidal active ingredients has become increasingly common,
53 threatening a “looming public health catastrophe” (6). Physiological resistance to pyrethroids,
54 the only class of insecticides suitable for use on LLINs, is now widespread and undermining the
55 impact of vector control all across Africa (7).

56 Only four directly lethal insecticide classes are currently recommended for control of adult
57 malaria vectors with LLINs or IRS: Pyrethroids (eg permethrin, deltamethrin, lambda-
58 cyhalothrin), organochlorines (eg DDT), carbamates (eg bendiocarb, propoxur) and
59 organophosphates (eg. malathion, fenitrothion, pirimiphos methyl) (8). Mechanisms of cross-

60 resistance against both organochlorines and pyrethroids limit their utility for combined use in
61 rotations, mosaics or combinations (7, 8). Organochlorines (DDT in particular) and carbamates
62 have a long history of use in both agriculture and public health and resistance to both these
63 classes is already emerging following only a few brief years of use in IRS at programmatic scales
64 (7). Neither these classes, nor the organophosphates, can be safely applied to LLINs at
65 operationally effective doses (8), and they are all prohibitively expensive for routine IRS
66 applications (9-11).

67 For example, year-round protection of all 40 million (M) people at risk in Tanzania, with IRS
68 using the ideal recommended dose of the new capsule suspension (CS) formulation of
69 organophosphate pirimiphos-methyl (PM), would cost \$157M annually for insecticide
70 procurement alone, exceeding the entire national malaria control budget of \$114. PM
71 procurement alone for continuous IRS coverage of all at-risk populations would cost \$3.3 Billion
72 (B) annually across Africa and \$12.5B worldwide, dwarfing the total global malaria control
73 budget of only \$2.5B (10). As such expensive insecticides have become increasingly necessary
74 due to pyrethroid resistance, IRS coverage has inevitably declined (9-11) and now stands at only
75 3.4% globally (12). While new insecticides are being developed for malaria vector control (6, 7,
76 13), these may well be similarly expensive. Also, unless these new active ingredients are astutely
77 delivered through rotations, mosaics or combinations, they may not necessarily be any less prone
78 to the emergence of physiological resistance (6-8).

79 Beyond physiological resistance, the impacts of LLINs and IRS are also attenuated by the
80 tendency of vectors to enter but then rapidly exit again from houses, without resting on treated
81 surfaces for long enough to accumulate a lethal doses of insecticide (14-16). Repeatedly entering
82 and then rapidly exiting from several houses, until an unprotected human victim can be attacked,

83 allows mosquitoes to mediate persistent residual malaria transmission, by maximizing their
84 feeding opportunities while minimizing their risks of exposure to LLINs and IRS when foraging
85 indoors (17, 18). New insecticide delivery methods will therefore be required to tackle such
86 evasive early-exiting vectors (14, 16), which may be described as behaviorally resilient (pre-
87 existing traits, typically with considerable phenotypic plasticity) or even resistant (increasing
88 frequency of selected heritable traits) (17, 19). In fact, life history simulation analyses suggest
89 such repeated visits to houses represent a vulnerability that can be exploited to great effect with
90 improved methods for killing mosquitoes inside houses (17, 18). Even for early-exiting vectors
91 which often feed outdoors instead, most mosquitoes old enough to transmit malaria have
92 previously entered at least one house, where they could be targeted with lethal insecticides or
93 traps (18).

94 The personal protection provided by LLINs and IRS can be superseded and improved upon by
95 physically mosquito-proofing houses with screened windows, ceilings and closed eaves (20).
96 However, most of the impact of LLINs and IRS upon malaria transmission is achieved by killing
97 off mosquito populations *en masse* to protect entire communities, with the more obvious
98 contributions of personal or household protection being far less equitable and important (4).
99 Household protection measures like spatial repellents or physical mosquito-proofing, which
100 merely deter mosquitoes from entering houses and force them to seek blood elsewhere, may
101 therefore have far less overall impact than those which kill them outright (21). In many settings
102 with highly efficient vectors, elimination of malaria transmission will probably require lethal
103 measures that suppress (3-5), or even eliminate (22), entire mosquito populations, rather than
104 merely deter them from entering houses (21). New insecticide delivery methods are therefore

105 urgently needed, to enable affordable deployment of multiple active ingredients, and more
106 effective targeting of early-exiting mosquitoes (6, 8, 13).

107 Here we describe a simple housing modification with widely-available netting materials, which
108 traps mosquitoes inside houses after they enter, and forces them into lethal contact with
109 insecticides when they attempt to exit again (Figure 1). Eave baffles have been used for decades
110 (23) in standardized experimental hut designs for assessing LLINs and IRS (24, 25). Eave baffles
111 consist of netting panels slanting inwards and upwards from the upper end of the wall towards
112 the roof, but leaving a small gap so that mosquitoes can freely enter the hut but cannot leave by
113 the same route (Figure 1A). Eave baffles have been successfully used to target house-entering
114 mosquitoes with fungal entomopathogens (26), so here they were combined with netting window
115 screens, and evaluated as a targeted delivery format for “off-the-shelf” formulations of
116 commonly-used chemical insecticides (Figure 1B). Even though treated window screens and
117 eave baffles (WSEBs) required far less insecticide than IRS, they achieved equivalent control of
118 physiologically-resistant *Anopheles funestus* and improved control of early-exiting *An.*
119 *arabiensis*. All these experiments were conducted in rural Tanzania with commercially-available
120 IRS formulations of pyrethroids and organophosphates, which were combined with existing
121 binding agent (BA) products for extending insecticide durability on LLINs.

122

123 **Methods**

124 These experiments were conducted in Lupiro village in the Kilombero Valley of southern
125 Tanzania, where intense malaria transmission is mediated by two of the most important malaria
126 vectors in Africa: (1) Local *Anopheles funestus* mediate *rebounding* (14) malaria transmission
127 because they are physiologically resistant to pyrethroids, carbamates and organochlorines (27),

128 and (2) Local *An. arabiensis* mediate resilient *residual* transmission (14) because they are
129 physiologically resistant to pyrethroids (27) and also exhibit early-exiting behaviors that render
130 them remarkably robust to indoor control with LLINs and IRS (18, 28, 29). All procedures were
131 approved by the Institutional Review Board of the Ifakara Health Institute (IHI/IRB/34-2014)
132 and the Medical Research Coordination Committee of the National Institute for Medical
133 Research (NIMR/HQ/R.8a/Vol IX/1903).

134 Thirteen experimental huts of the Ifakara design (24, 29, 30) were used to assess the impact of
135 LLINs, IRS and insecticide-treated WSEBs, using standard methodology (31). Four of these huts
136 were randomly selected and their inner wall and roof surfaces were sprayed with $2 \text{ g}\cdot\text{m}^{-2}$ of a CS
137 formulation of PM (Actellic 300CS®), using standard programmatic application procedures (32).
138 Another four randomly-selected huts were sprayed with $30 \text{ mg}\cdot\text{m}^{-2}$ of the pyrethroid lambda-
139 cyhalothrin (LC), also in a CS formulation (Icon 10CS®). The remaining five huts were sprayed
140 only with water to act as negative controls. Both of these long-lasting micro-encapsulated
141 insecticide formulations are manufactured by Syngenta Crop Protection AG, Basel Switzerland
142 for IRS applications, and are well characterized (33-35). After spraying, two mattresses and fully
143 intact Permanet™ LLINs (100 denier polyester multifilament mesh with $156 \text{ holes}\cdot\text{inch}^{-2}$,
144 surface-treated with $45 \text{ to } 55 \text{ mg}\cdot\text{m}^{-2}$ of deltamethrin in a resin foundation) were installed in each
145 hut.

146 Eave baffles are incorporated into experimental hut designs, to ensure mosquitoes can enter
147 through approximately half of the eave gaps between the wall and the roof, but are then all either
148 retained in the hut itself or forced into interception traps fitted to the remaining exit points (24,
149 25). In a conventional experimental hut study, those remaining exit points are the windows and
150 the remaining un-baffled half of the eave gaps (24, 25). However, the purpose of this study was

151 to evaluate WSEBs as an insecticide delivery format in their own right. All the WSEB
152 treatments, except for the full negative control, therefore included eave baffles fitted to *all* eave
153 gaps, with and without exit traps, and identically-treated screens fitted over *all* windows (Table
154 1, Figure 1). Treated WSEBs were fitted in front of the exit traps, which were fitted immediately
155 outside the hut (24), so that any mosquito attempting to exit through any eave gap or window
156 would be forced into contact with these insecticidal netting barriers (Figure 1).

157 The only treatment without screens over the windows, or eave baffles over the half of the eave
158 gaps with exit traps immediately outside, was therefore the full negative control (Table 1). These
159 full negative controls had untreated eave baffles fitted only to the half of the eave spaces lacking
160 exit traps, thus allowing mosquitoes to both enter and exit. The two partial negative controls had
161 screens fitted over the windows and baffles fitted to all eave gaps, regardless of whether they
162 acted as entry or exit points for mosquitoes, but were not treated with any insecticides (Table 1).
163 One of the partial negative controls was treated with the non-insecticidal binding agent (BA) that
164 Syngenta include along with LC (the same Icon 10CS formulation we used for IRS) in their Icon
165 Maxx® product, to extend its active life on polyester netting (36). Note that although the
166 manufacturer-recommended dose of LC on netting treated with the Icon Maxx® product (55
167 mg·m⁻²) is somewhat higher than used for IRS (30 mg·m⁻²), it is similar to that for deltamethrin
168 on the Permanet® LLINs used in this study (45 to 55 mg·m⁻²).

169 The first insecticidal WSEB treatment, listed fourth in Table 1, was this same long-lasting Icon
170 Maxx® product, this time including both the BA and the LC active ingredient (36). Also,
171 WSEBs treated with PM were assessed at three different dosages that were comparable with
172 typical IRS application rates per square meter treated (Table 1). These three PM doses were also
173 assessed as a co-treatment with BA to potentially extend insecticide life, both with and without

174 LC as a complementary second insecticide from a different chemical class (Table 1). LC was
175 chosen, despite coming from the pyrethroid class to which both vector species in the study area
176 are resistant (27), to assess the potential of such cocktails to select for restored pyrethroid
177 susceptibility by selectively reducing mortality of insects that are both susceptible to its lethal
178 mode of action and responsive to its irritant/repellent effects on mosquito behaviour (37). The
179 mathematical modeling study which motivated assessment of this combination assumed that
180 these two pyrethroid susceptibility and responsiveness phenotypes, and presumably their
181 underlying genotypes, are closely associated and therefore co-selected (37).

182 While all exit traps on eaves and windows were made of Teflon-coated fibreglass mesh (24), all
183 eave baffles and window screens were instead made of 100-denier polyester netting (A to Z
184 Textile Mills, Arusha, Tanzania) of the kind typically used for bed nets. All WSEB were treated
185 by soaking in aqueous suspensions of the insecticides and/or BA and then drying in the shade.

186 To execute the full experimental design of this study, duplicate sets of the 13 detachable,
187 movable WSEB treatments (Table 1), were rotated nightly through the 13 huts over two full 26-
188 day rounds of experimental replication (Additional file 1), between the 5th of December 2015 and
189 the 1st of February 2016. Each night, two adult male volunteers slept under the two LLINs inside
190 each hut from 19:00 to 07:00 hr. The volunteers then collected all mosquitoes inside the hut with
191 a Prokopak aspirator (John W. Hock) (38), and then those inside the exit traps with a mouth
192 aspirator (24). Dead mosquitoes were then sorted taxonomically, classified by sex and abdominal
193 status, and counted. Specimens collected alive were maintained in a field insectary for 24 hours
194 before separating live and dead specimens for sorting, classification and counting. A random
195 sample of 242 specimens from the *An. gambiae* complex were identified to sibling species by
196 polymerase chain reaction (39).

197 Each pair of volunteers remained assigned to a fixed experimental hut throughout the study, so
198 that variability associated with these individuals and the huts themselves could be analyzed as a
199 single, consistent source of variance. Following mosquito collection each morning, each pair of
200 volunteers was only responsible for installing the set of WSEBs assigned to their hut that
201 evening, and for removing it from the hut it had been fitted to the previous night. All volunteers
202 used a fresh pair of gloves each morning and were not allowed to handle any WSEBs other than
203 those to be used in their hut that night. All WSEB sets were individually labelled, and stored in
204 labelled buckets during transfer between huts and the 13 day storage period of each 26 day
205 replication cycle (Additional file 1).

206 All field data were collected on hard copies of the ED1 and SS3 forms, recently described for
207 informatically-robust collection of entomological data (40). To ensure rigid compliance with the
208 experimental design, all attributes defined by it were prefilled into the forms (Additional file 1).
209 All statistical analysis was accomplished using generalized linear mixed models (GLMMs) with
210 a binomial distribution and logit link function for the binary mosquito mortality outcome, fitted
211 using R version 3.2.1. The IRS and WSEB treatments were included as categorical independent
212 variables, while hut and night were included as random effects.

213

214 **Results**

215 A total of 1318 specimens from the *An. funestus* group and 5842 from the *An. gambiae* complex
216 were captured. Molecular identification in the laboratory confirmed the continued absence of
217 nominate *Anopheles gambiae* from the study area (22), with all (100%; 176/176) successfully
218 amplified (73%; 176/242) specimens from this complex identified as *An. arabiensis*. All

219 WSEBs, other than the full negative control, clearly retained mosquitoes within the huts, because
220 this is where the vast majority (>90%) were collected, rather than in the exit traps.

221 ***Comparing the impact of WSEBs and IRS upon An. funestus mortality***

222 When used alone, most of the WSEB treatments that included insecticides (8/10) killed similarly
223 high proportions of *An. funestus* to IRS alone using the same insecticide formulations (Figure
224 2A). For example, mortality for LC plus BA-treated WSEBs alone was indistinguishable from
225 LC IRS alone ($P=0.363$). The only exceptions amongst the 10 WSEB treatments were those two
226 with the highest PM dose plus BA and the intermediate PM dose plus LC and BA: Both of these
227 WSEB treatments alone killed somewhat lower proportions of *An. funestus* than IRS with LC
228 alone, and a similar but non-significant pattern was observed for comparisons of the same WSEB
229 treatments alone with PM IRS alone (Figure 2A, Additional file 2). Nonetheless, mortality rates
230 achieved by PM-treated WSEBs alone were consistently high (Figure 2A), regardless of
231 treatment dosage ($P\geq 0.156$), and were statistically indistinguishable from PM IRS alone
232 ($P\geq 0.713$), even though the lowest WSEB dose per unit area treated was only half that for IRS.
233 While all the combinations of PM-treated WSEBs with PM IRS resulted in higher mortality than
234 PM-IRS alone or PM-treated WSEBs alone, none of these contrasts were significant ($P\geq 0.080$)
235 because too few mosquitoes survived either the treated WSEBs alone or IRS alone.

236 ***Comparing the impact of WSEBs and IRS upon An. arabiensis mortality***

237 Overall, insecticide-treated WSEBs either matched or proved superior to IRS when deployed
238 against *An. arabiensis* (Figure 2B, Additional file 2). WSEBs alone treated with LC plus BA
239 achieved similar mortality to IRS alone with the same LC formulation ($P=0.345$). WSEBs alone
240 treated with the lowest dose of PM achieved similar *An. arabiensis* mortality to IRS alone using
241 twice as much PM per square meter treated ($P=0.419$). However, increasing the PM treatment

242 dosage from 1 to 2 or 4 g·m⁻² increased the mortality achieved by WSEBs alone (OR [95%CI]
243 =2.10 [1.16, 3.79], P = 0.0139 and 2.34 [1.28, 4.26], P = 0.0055, respectively), although there
244 was no difference between the intermediate and high dosages (P=0.758). WSEBs alone with
245 either the intermediate or high PM dosage killed more *An. arabiensis* (Odds ratio (OR) [95%
246 Confidence Interval (CI)] = 5.9 [1.4, 24.3], P=0.0145 and 10.8 [1.6, 74.8], P=0.0157,
247 respectively) than IRS alone, even though the intermediate PM dosage was the same as IRS per
248 square meter treated. Supplementing PM-treated WSEBs with PM IRS did increase *An.*
249 *arabiensis* mortality for the lowest WSEB dose (OR [95% CI] = 4.8 [1.5, 15.5], P=0.0081),
250 which was half that of IRS per unit area treated. However, supplementary PM IRS did not
251 increase mortality when WSEBs were treated with the same dosage as IRS (P=0.748), or with
252 twice that dosage (P=0.429).

253 ***Combining PM with BA and LC as WSEB co-treatments***

254 Adding BA had no effect on the mortality rates achieved by PM-treated WSEBs alone, for either
255 *An. funestus* (P = 0.393) or *An. arabiensis* (P = 0.424). Supplementing the organophosphate PM
256 plus BA treatment with the irritant pyrethroid LC as a second active ingredient, reduced *An.*
257 *funestus* mortality rates achieved by WSEBs alone (OR [95% CI] = 0.64 [0.46, 0.89], P =
258 0.0076), presumably because the irritant properties of LC reduce mosquito contact times with co-
259 treated WSEBS, and therefore exposure to both insecticides. A similar but less dramatic and non-
260 significant trend was observed for *An. arabiensis* (OR [95% CI] = 0.88 [0.73, 1.06], P = 0.174).

261

262

263 **Discussion**

264 While WSEBs exhibited higher efficacy than IRS against early-exiting *An. arabiensis*, the two
265 delivery formats had similar efficacy against *An. funestus*. The most striking advantage of
266 WSEBs is therefore that it reduced the surface area treated per hut by more than 5-fold.
267 Furthermore, the possibility of co-application with existing BAs that already extend durability of
268 pyrethroids on LLINs (36) for up to 3 years (41), suggests new opportunities for also reducing
269 reapplication frequency by up to six-fold, relative to IRS.

270 Of course these WSEBs are merely an experimental prototype, which were evaluated in the
271 necessarily homogenous and controlled environment of experimental huts. This short term
272 efficacy study cannot address key issues regarding the potential effectiveness and cost-
273 effectiveness of WSEBs under programmatic operational conditions. It is encouraging that a full
274 set of these WSEBs for these experimental huts, specifically designed to match the dimensions
275 of local houses (24), required only 11 m² of netting to manufacture, similar to a typical LLIN.
276 However, they had to be carefully hand-tailored with hooks and Velcro™ to enable easy daily
277 removal and reinstallation in experimental huts, at a manufacturing labor cost of \$47 per set.
278 More practical and affordable formats for operational use in a diversity of house designs clearly
279 to be developed and rigorously evaluated before WSEBs could be considered for routine,
280 programmatic deployment by national programs.

281 Nevertheless, the potential of this approach merits consideration, even if only speculatively at his
282 early stage. For example, it takes almost an entire 833ml bottle of the 0.3 g·ml⁻¹ PM formulation
283 used here, costing almost \$24, to protect just one typical rural Tanzanian house against perennial
284 transmission for one year, through two IRS treatments of its internal surfaces (60 m² (24)) at the
285 ideal recommended dose of 2 g·m⁻². By comparison, a house of equivalent size with WSEBs

286 installed could be treated with the same insecticide at the same dosage per square meter of
287 treated netting for only \$2.15. While greater quantities of BA may be required than applied here
288 (42), if it were to extend the life of PM on netting to the same extent as LC, and the physical
289 structure of WSEBs themselves were also to last that long in real houses under normal
290 operational conditions, they could potentially provide up to 3 years of protection for only \$0.72
291 per annum in recurrent insecticide procurement costs. Scale up nationally in Tanzania would cost
292 only \$4.8M for insecticide procurement, so even a combination of three similarly expensive
293 complementary insecticides would be affordable to the national program at <\$15M annually.
294 Corresponding global costs would be <\$1.2B annually for such a triple cocktail.

295 While these insecticide cost estimates are entirely speculative, assume that BA will be equally
296 efficacious for extending the longevity of PM, and do not consider costs of netting installation or
297 maintenance, they do illustrate the potential economic benefits that could be accrued by
298 optimizing WSEB deployment formats, netting materials and treatment formulations. More
299 importantly, such reduced insecticide requirements might make rational resistance management
300 (8) both feasible and affordable with existing budgets and off-the-shelf insecticide products.

301 Also, the observation that supplementing PM-treated WSEBs with the irritant pyrethroid LC
302 reduced mortality rates of *An. funestus*, which were strongly resistant to pyrethroids but not
303 organophosphates (27), suggests WSEBs could be used as an affordable format with which to
304 field-test the theory that such combinations might select for restored pyrethroid susceptibility
305 (37). The underlying assumption of this hypothesis is that physiological susceptibility and
306 behavioral responsiveness to pyrethroids are genetically linked, so that insecticide combinations
307 like the LC-PM mixture used here would selectively kill insects that are both resistant and non-
308 responsive to pyrethroids. The case for assuming physiological susceptibility and behavioral

309 responsiveness are at least phenotypically associated has recently been strengthened by
310 laboratory studies of *Culex quinquefasciatus*, demonstrating that four different pyrethroid-
311 resistant field populations were all less responsive to the irritant properties of permethrin than a
312 fully-susceptible laboratory colony (43). These empirical studies (43) also suggest grounds for
313 optimism regarding the recent theory that combining recently-developed low-technology
314 emanators for airborne pyrethroid vapor (44, 45) with complementary non-pyrethroid indoor
315 control measures like IRS, WSEBs or alternative technologies such as eave tubes (46-48) and
316 entry traps (49), could also co-select for physiological susceptibility and behavioral
317 responsiveness to pyrethroids generally (50). Nevertheless, genetic linkage between
318 physiological susceptibility and behavioural responsiveness to pyrethroids remains to be
319 demonstrated. Also, both of the mathematical models predicting restoration of these preferred
320 traits (37, 50), by definition, merely illustrate the plausibility of these hypotheses in
321 mathematically explicit terms. Alternatively, it is also possible that selection for physiological
322 resistance to both insecticides may be exacerbated by reducing contact exposure to sub-lethal
323 levels. So while the potential benefits and risks of combining irritant pyrethroids with non-
324 irritant insecticides from complementary classes remain to be satisfactorily assessed, the results
325 presented here suggest that WSEBs may be a potentially scalable delivery format with which to
326 test these hypotheses empirically through large-scale field studies.

327 Changing deployment format for existing IRS formulations could also eliminate the need to
328 apply them in potentially hazardous aerosol form. While handling insecticides is always
329 associated with some risks, so protective clothing, eyewear and breathing apparatus might be
330 required, WSEBs may be impregnated by simply dipping in an aqueous suspension, similarly to

331 bed nets. WSEB deployment formats might therefore allow national programs to develop and
332 manage their vector control platforms more flexibly than IRS.

333

334

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341 **Biographical Sketch**

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346 and institutional levels.

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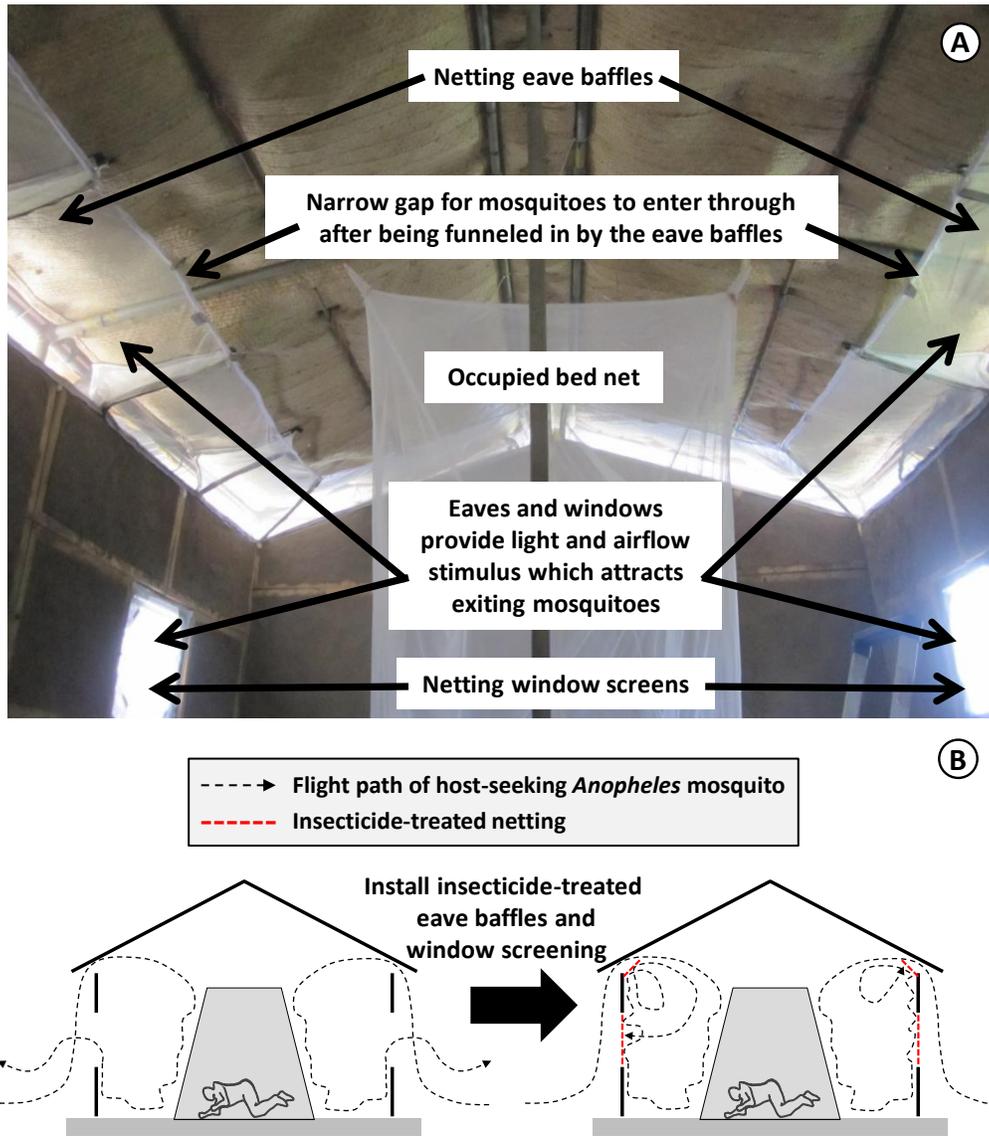
477 **Additional file 1.** The 26-day schedule applied to complete one full replicate of evaluation for
478 duplicates of the 13 treatments of window screens and eave baffles (WSEBs), by rotating them
479 through all of the 13 pre-sprayed experimental huts.

480

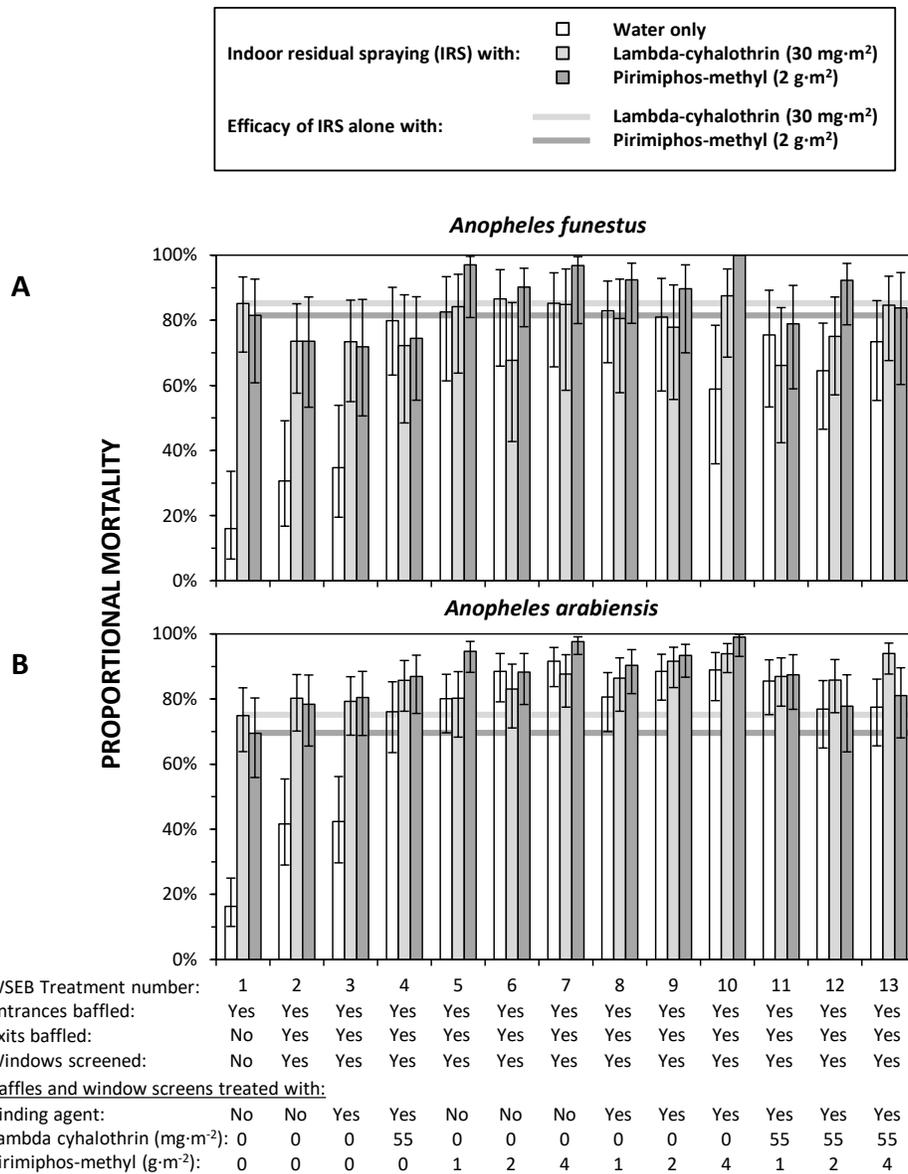
481 **Additional file 2.** Detailed tables describing the estimated mortality rates of both malaria vector
482 species in houses with each of the 39 combinations of treatments for indoor residual spraying
483 (IRS) and window screens plus eave baffles (WSEB), as well as the odds ratios, 95% confidence
484 intervals and significance levels for contrasts between each of these WSEB treatments alone and
485 each other and either of the IRS treatments alone.

486 **Table 1.** A summary of the thirteen different window screening and eave baffle (WSEB) treatments which were rotated through
487 experimental huts with three different indoor residual spray (IRS) treatments. IRS treatments of the experimental huts comprized
488 either lambda-cyhalothin (LC: 30 mg·m⁻² in 4 huts), pirimiphos-methyl (PM: 2 g·m⁻² in 4 huts), or the negative control (Water diluent
489 only: 5 huts), applied to all inner surfaces of the walls and ceilings. Note that all dosages described herein are per square meter of
490 treated netting (WSEBs) or wall and ceiling surface (IRS), so that these can be directly compared in terms of lethality and cost per unit
491 area treated. The 26-day schedule applied to complete one full replicate of evaluation for duplicates of these 13 treatments, by rotating
492 them through all 13 IRS-treated experimental huts, is detailed in additional file 1.

Number	Description	Eaves baffled		Windows screened	Treatment of window screen and eave baffle (WSEB) netting		
		Entrances	Exits		Lambda-cyhalothrin (LC: mg·m ⁻²)	Pirimiphos-methyl (PM: g·m ⁻²)	Binding Agent (BA)
1	Full negative control: No trapping or insecticide	Yes	No	No	0	0	No
2	Partial negative control: Trapping without insecticide	Yes	Yes	Yes	0	0	No
3	Partial negative control: Trapping without insecticide	Yes	Yes	Yes	0	0	Yes
4	Trapping plus long-lasting LC+BA treatment	Yes	Yes	Yes	55	0	Yes
5	Trapping plus varying dose PM treatments	Yes	Yes	Yes	0	1	No
6		Yes	Yes	Yes	0	2	No
7		Yes	Yes	Yes	0	4	No
8	Trapping plus varying dose PM treatments with BA	Yes	Yes	Yes	0	1	Yes
9		Yes	Yes	Yes	0	2	Yes
10		Yes	Yes	Yes	0	4	Yes
11	Trapping plus varying dose PM treatments with BA+LC	Yes	Yes	Yes	55	1	Yes
12		Yes	Yes	Yes	55	2	Yes
13		Yes	Yes	Yes	55	4	Yes



495 **Figure 1.** Design (A) and mechanism of action (B) of insecticide-treated window screens and
496 eave baffles (WSEBs).



498

499 **Figure 2.** Impact of window screens and eave baffles (WSEBs) treated with various
500 combinations of insecticides and binding agents (Table 1) upon malaria vector mortality inside
501 experimental huts, which were previously sprayed with one of three alternative indoor residual
502 spraying (IRS) regimens (Additional file 1), and occupied by two volunteers sleeping under
503 pyrethroid-treated long-lasting insecticidal nets (LLINs). Each of these estimated mean mortality
504 rates and confidence intervals, as well as the statistical contrasts between the most relevant
505 treatment pairs, are presented in explicit numerical format in Additional file 2.