Host-seeking behaviour of *Anopheles* mosquitoes in response to olfactory and visual cues

Margaret Mendi Njoroge

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Propositions

1. An odour-baited trap in close proximity of the eave diminishes the effectiveness of the repellent transfluthrin applied on eave fabric in reducing the number of outdoor-biting mosquitoes in the peri-domestic area.
   (this thesis)

2. In the presence of potent spatial repellents, mosquito species differ in how they respond to attractive synthetic lures.
   (this thesis)

3. Application of agricultural pesticides increases the risk of schistosomiasis.

4. Aquaculture leads to reduction in natural fish populations in freshwater bodies.

5. Political affiliations diminish knowledge acquisition and application.

6. Accessibility to affordable internet services increases the risk of under-age pregnancies.

Propositions belonging to the thesis, entitled:

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General Introduction

Chapter 1

Introduction

Success in malaria vector control in recent years has been attributed largely to the widespread distribution and utilization of long lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS)(WHO, 2019). The disruption of contact between the human host and the vector remains a very effective mechanism of controlling mosquito-borne diseases when applied in combination with other tools such as effective diagnosis, prevention of mother to child transmission and prompt medical treatment (WHO, 1975; Lindsay *et al.*, 2002; Russell *et al.*, 2011; Bhatt *et al.*, 2015b; WHO, 2019).

While LLINs and IRS have contributed to the reduction in malaria disease burden, the widespread emergence of mosquitoes resistant to pyrethroids, the main insecticide group used on LLINs and IRS, and other insecticides threatens the continued success of these tools (Townson *et al.*, 2005; Tanner *et al.*, 2008; Sande *et al.*, 2015; Awolola *et al.*, 2018; Cook *et al.*, 2018; Deletre *et al.*, 2019; WHO, 2019; Hancock *et al.*, 2020). Increased pyrethroid resistance of mosquitoes in malaria endemic areas prompted the modification of formulations used on LLINs to include synergists such as piperonyl butoxide (PBO) that inhibit the enzymes that detoxify insecticides and have increased the effectiveness of existing insecticides (LeClair *et al.*, 2017; Matowo *et al.*, 2017; Gleave *et al.*, 2018; Rakotondranaivo *et al.*, 2018; Birhanu *et al.*, 2019). This solution, however, is considered to be a delay and not a preventive measure to the spread of insecticide resistance, with studies already showing decreasing efficiency of synergist-included insecticide formulations on bed nets (Djouaka *et al.*, 2016; Gleave *et al.*, 2018; Riveron *et al.*, 2019).

Another challenge in malaria control is the apparent shift of dominant malaria vector populations from predominantly indoor-biting and resting populations to those that rest and bite outdoors and/or bite earlier in the night when people are not yet protected by LLINs (Antonio-Nkondjio *et al.*, 2006; Moore *et al.*, 2012; Moiroux *et al.*, 2014; Sougoufara *et al.*, 2014; Moshi *et al.*, 2017; Limwagu *et al.*, 2019; Mburu *et al.*, 2019; Sherrard-Smith *et al.*, 2019). This change in vector behaviour is expected to increase the likelihood of receiving an infectious bite during the evening while conducting outdoor activities, before retiring indoors where protection from either LLINs or IRS is present (Killeen, 2014; Mathania *et al.*, 2016; Moshi *et al.*, 2017). Studies have shown that socio-economic activities such as fishing, cooking and harvesting in rural areas are associated with an increased risk of exposure to outdoor malaria transmission (Sande *et al.*, 2015; Moshi *et al.*, 2017; Moshi *et al.*, 2018). The need to identify alternative tools that can be applied promptly towards management of physiologically and behaviourally resistant outdoor-biting mosquitoes is urgent to further reduce malaria incidence (Sangoro *et al.*, 2014a; Sherrard-Smith *et al.*, 2019).

Mosquitoes utilize very specific chemical and physical cues when conducting various physiological functions such as mating (Vaníčková *et al.*, 2017), location of an ideal breeding habitat (Okal *et al.*, 2013; Lindh *et al.*, 2015; Asmare *et al.*, 2017), identification of a plant for sugar feeding (Foster, 2008; Nyasembe *et al.*, 2012; Nyasembe *et al.*, 2018) and the successful location of a host for a blood meal (Takken *et al.*, 1997; Takken *et al.*, 1999; Carde, 2015; Liu *et al.*, 2019). Understanding the very complex mechanisms involved in host-seeking (Hawkes *et al.*, 2016; Hawkes *et al.*, 2017) is key to developing tools that manipulate mosquitoes to move away from potential human hosts and on to non-human hosts (Donnelly *et al.*, 2015; Njoroge *et al.*, 2017) or to trap and kill devices that mimic human cues (Takken *et al.*, 1999; Mukabana *et al.*, 2002; Qiu *et al.*, 2007; Olanga *et al.*, 2010). Mosquito perception of olfactory cues takes place in receptors located on antennae and maxillary palps which inform the mosquito of the potential availability of the blood meal host prior to utilization of complementary cues of physical nature, *i.e.* temperature, moisture, and air speed, to guide landing and contact chemical cues perceived upon landing on the skin, in particular tastants, that elicit feeding (Takken *et al.*, 1999; Turner *et al.*, 2011; Webster *et al.*, 2015; Raji *et al.*, 2017).

Repellents are chemical compounds that have been in use since antiquity to deter biting insects away from humans usually by interfering with the normal perception and response of insects to humans (Charlwood, 2003; Paluch *et al.*, 2010; Maia *et al.*, 2011; Maia *et al.*, 2018). Usually applied either topically, on clothes or as volatile or spatial chemicals, repellents provide opportunities for controlling malaria (Wilson *et al.*, 2014; Maia *et al.*, 2018). Topical repellents exert their effect on their targets once direct interaction of mosquitoes with treated areas takes place (Norris *et al.*, 2017; Dennis *et al.*, 2019) and are characterized as being only slightly volatile which extends their effective action from a treated surface (usually: the skin)(Grieco *et al.*, 2007; Sathantriphop *et al.*, 2014). Insecticide treated clothes have been shown to reduce the risk of malaria by 50% and can provide protection in areas where LLINs cannot be in use such as in disaster areas (Maia *et al.*, 2018). Volatile or spatial chemicals represent a promising solution in reducing the spread of mosquito-borne disease due to their ability to disrupt normal host-vector interactions within a particular area (Achee *et al.*, 2012; Sangoro *et al.*, 2014a; Norris *et al.*, 2017; Bowman *et al.*, 2018; Moshi *et al.*, 2018).

Some insecticides have lethal, sub-lethal and excito-repellent properties though the effects of these in vector control are understudied (Chareonviriyaphap *et al.*, 2013). In the face of insecticide resistance, vectors may respond less to toxicity of insecticides due to innate mechanisms that reduce the toxic effects but may still respond to sub-lethal and excito-repellent effects of the same insecticides (Guedes *et al.*, 2017; Cook *et al.*, 2018; Thievent *et al.*, 2019). Sub-lethal concentrations of insecticides present opportunities for application as they reduce host-vector contact even when mosquito populations are predominantly resistant to that group of insecticides (Cohnstaedt *et al.*, 2011; Bowman *et al.*, 2018). Recently, however, concerns have been raised on how lower insecticide concentrations may still select populations that are more resistant by either increasing fitness or selecting a wide-range of resistance-linked genes that will increase dominance in the population (Guedes *et al.*, 2017; Margus *et al.*, 2019). Insect repellents act by diverting mosquitoes away from attractive targets, thereby interfering with responses to the attractive cues emanated by the host. Repellency reduces vector-host contact and subsequently diminishes the chances of transmission of parasites and viruses to human (Norris *et al.*, 2017).

Results from field studies testing topical repellents show differences in outcome and protection (Maia *et al.*, 2018). In a study done in Senegal, the effect of topical repellents (Para-menthane-3,8-diol (PMD), DEET and icaridine) on the biting rates of mosquitoes was shown to be significant in providing protection on exposed human subjects compared to the control groups when mosquito biting rates were monitored for nine hours after application (Uzzan *et al.*, 2009). However, a study in Tanzania showed that 15% DEET used as a topical repellent in the early evenings was not effective in reducing malaria prevalence (Sangoro *et al.*, 2014a; Sangoro *et al.*, 2014b). The reasons for this were attributed to lack of compliance in application; both in technique and in frequency therefore rendering what could have been an effective tool in preventing host-vector contact to be ineffective for disease prevention and control in this setting (Sangoro *et al.*, 2014a). Frequent re-application of a topical repellent can be impractical or impossible in areas where supplies are limited or where there is lack of proper education and training on proper use. In some cases, even with adequate education, adherence to application regime is low, therefore reducing effectiveness of contact repellents (Sangoro *et al.*, 2014a; Lalani *et al.*, 2016). Moreover, in many tropical areas where mosquito-borne diseases are endemic, high levels of perspiration remove the repellent from the skin, making this approach less effective in the long-run (Barnard, 2005; Wilson *et al.*, 2014; Norris *et al.*, 2017).

Use of spatial repellents instead of topical repellents reduces the impracticality of regular application in favour of active or passive emanation leading to continued action with minimal labour input (Achee *et al.*, 2012; Lynch *et al.*, 2016; Norris *et al.*, 2017). Spatial repellent compounds are highly volatile and capable of diffusing through the air and acting on targets at a distance from the point of release (Achee *et al.*, 2012; Norris *et al.*, 2017) making them ideal for use both indoors and outdoors (Grieco *et al.*, 2007; Lynch *et al.*, 2016; Norris *et al.*, 2017). The vaporization of these compounds, either passively or through the use of a fan or heat-based emanator, creates saturated pockets of air which induce an aversive behaviour from the malaria vectors (Achee *et al.*, 2012; Ogoma *et al.*, 2014b). Compounds such as transfluthrin, delta-undecalactone, dichlorodiphenyltrichloroethane (DDT) among many others have been shown to have a spatial repellent effect on malaria mosquitoes (Barasa *et al.*, 2002; Pates *et al.*, 2002; Grieco *et al.*, 2007; Menger *et al.*, 2014; Ogoma *et al.*, 2014b; Sangoro *et al.*, 2014b; Deletre *et al.*, 2019). Transfluthrin has been mostly used indoors and has either been applied on surfaces or fabric, evaporated from heated devices or burning coils, or dispensed by devices with fans (Ogoma *et al.*, 2014b; Menger *et al.*, 2016; Ogoma *et al.*, 2017). Interestingly, some insecticides such as transfluthrin and chlorpyrifos methyl exhibit spatial repellent tendencies at sub-lethal concentrations (Achee *et al.*, 2009; Ogoma *et al.*, 2014b; Ogoma *et al.*, 2017) while other insecticides such as DEET have a stronger repellent effect than a toxic or killing effect on mosquitoes (Dennis *et al.*, 2019).

Many spatial repellents that have been developed for market use have been in the form of electrical vaporisers or mosquito coils (Ramesh *et al.*, 2001; Pates *et al.*, 2002; Nazimek *et al.*, 2011; Maia *et al.*, 2018). As many malaria endemic areas in rural Africa lack electricity, mosquito coils have been more commonly used, though their contribution to environmental pollution and respiratory illness have led to public health calls for their discontinued use (Hogarh *et al.*, 2016; Madhubabu *et al.*, 2017; Hogarh *et al.*, 2018; Tangena *et al.*, 2018).

Alternative methods of availing spatial repellents have been considered and suggestions made for the use of treated outdoor decorations, containers and eave fabrics particularly when produced using locally available fabrics such as hessian (burlap) fabric (Ogoma *et al.*, 2012b; Masalu *et al.*, 2017; Ogoma *et al.*, 2017; Masalu *et al.*, 2018). Many rural houses in Africa have open eaves as part of the house design to increase air circulation. These open eaves greatly contribute to increased risk of house-entry by malaria vectors and open eaves rank highly as a risk factor for malaria transmission (Ghebreyesus *et al.*, 2000; Njie *et al.*, 2009; Jatta *et al.*, 2018b; Kaindoa *et al.*, 2018; Mburu *et al.*, 2018). Screening of exposed or open eaves, even with untreated materials, has been shown to play a major role in the reduction in indoor mosquitoes in various studies (Lindsay *et al.*; Ogoma *et al.*, 2010; Menger *et al.*, 2016; Mburu *et al.*, 2018). Addition of insecticides on the eave fabric further reduces house entry behaviour of mosquitoes by up to 99% (Menger *et al.*, 2016). Using repellent-treated eave fabric on such houses can greatly reduce the number of host-seeking mosquitoes both indoors and outdoors through effective passive release yet without requiring separate electrically powered devices (Ogoma *et al.*, 2012b; Govella *et al.*, 2015; Masalu *et al.*, 2017; Ogoma *et al.*, 2017; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019). It would be, however, interesting to explore the possibility of using a treated eave strip or ribbon as opposed to an eave screen for consideration to use as the push component in the proposed push-pull strategy. An eave strip would utilize less fabric than full eave screening with fabric and would therefore reduce the initial costs to be incurred in the production of a push component for field application. Application in the field would be simpler than a full eave screen. Additionally, the variability of eave width in rural homes in western

Kenya would make it impossible to obtain a fabric that would cover all eaves as had been done in previous studies (Ogoma *et al.*, 2010; Menger *et al.*, 2016).

Application of spatial repellents has shown success in reducing host-vector interaction among protected hosts, although it is possible to unintentionally redirect mosquitoes to homesteads and persons that are not protected by a repellent (Maia *et al.*, 2016). To reduce this diversionary effect of spatial repellents on malaria vectors from protected populations to unprotected populations, a supplementary system could be added that would reduce the biting fraction of the mosquitoes by removal trapping thus reducing disease transmission (Cook *et al.*, 2007; Wagman *et al.*, 2015b).

The use of outdoor bait stations (traps or landing boxes) has been proposed in combination with spatial repellents in a push-pull system (Takken, 2010; Menger *et al.*, 2015; Wagman *et al.*, 2015b; Menger *et al.*, 2016). This concept proposes that the application of both push (spatial repellent) and pull (odour-baited stations) systems offers greater protection against mosquito bites than repellents alone or traps alone (Dekker *et al.*, 2001; Njiru *et al.*, 2006; Okumu *et al.*, 2010c; Revay *et al.*, 2013; Obermayr *et al.*, 2015) especially in the peridomestic area (*i.e.* the area in the direct circumference of a house; Figure 1) (Menger *et al.*, 2015). Once mosquitoes come into contact with spatial repellents, they are diverted away from the target area (Menger *et al.*, 2015). As they fly away from the repellent plume, mosquitoes perceive synthetic lures set up in either odour-baited traps or landing boxes and are attracted to them (Takken *et al.*, 1999; Njiru *et al.*, 2006; Qiu *et al.*, 2007; Okumu *et al.*, 2010b; Okumu *et al.*, 2010c; Mukabana *et al.*, 2012b; Menger *et al.*, 2015).

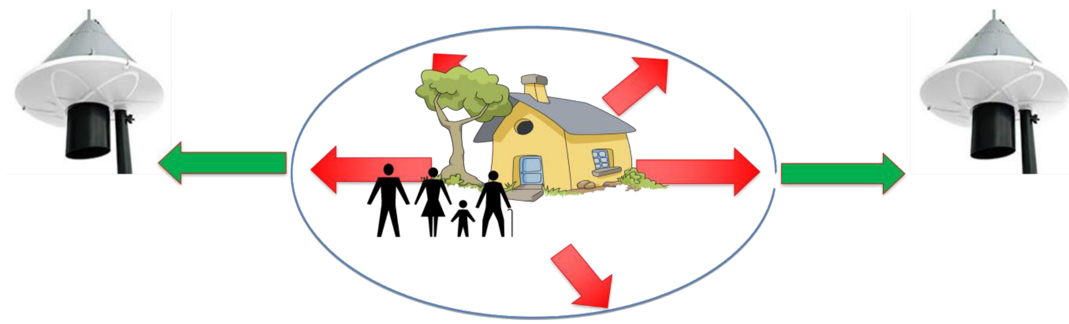

Figure 1. Proposed application of protection in the peridomestic area (encircled in blue) by the combination of push (red arrows), provided by the spatial repellent, and pull (green arrows), provided by odour-baited trap.

A key attractant used by mosquitoes to identify vertebrate hosts is carbon-dioxide (Takken *et al.*, 1989; Costantini *et al.*, 1996; Gillies, 2009). Traps utilizing attractive lures usually incorporate CO_2 for increased effectiveness (Gillies *et al.*, 1987; Takken *et al.*, 1989; Costantini *et al.*, 1996; Mboera *et al.*, 1997; Takken *et al.*, 1997; Mboera *et al.*, 2000; Gillies, 2009; Smallegange *et al.*, 2010; Mweresa *et al.*, 2014). When used alone or with odour lures, CO_2 activates host-seeking mosquitoes and induces them to fly upwind toward the trap. The odour plumes alone are not, however, sufficient to induce a mosquito to enter a trap, necessitating powered fans which suck mosquitoes into the trap (Cardé *et al.*, 2010a; Hiscox *et al.*, 2014). Synthetic lures usually play a major role in close-range attraction and landing (Takken *et al.*, 1997; Gibson *et al.*, 1999; Takken *et al.*, 1999; Dekker *et al.*, 2001; Foster *et al.*, 2004).

Provision of carbon dioxide can be costly as it requires special packaging of dry ice or metal tanks containing carbon dioxide under high pressure. To overcome this obstacle, an alternative source of carbon dioxide can be fermentation of sugar or molasses with yeast (Saitoh *et al.*, 2004; Oli *et al.*, 2005; Patrascu *et al.*, 2009; Smallegange *et al.*, 2010; Mweresa *et al.*, 2014). While designing the pull component of a push-pull product, it is crucial to explore possibilities of obtaining a carbon dioxide replacement for ease of field application due to the impracticality of generating sufficient carbon dioxide in the field (Oli *et al.*, 2005; Mburu *et al.*, 2017). One chemical that has been proposed as a possible alternative to carbon dioxide in attracting host-seeking malaria mosquitoes is 2-butanone (Mburu *et al.*, 2017), which was reported to activate the cpA neuron on the maxillary palp; in mosquitoes this olfactory receptor is the principal ligand of carbon dioxide (Lu *et al.*, 2007; Tauxe *et al.*, 2013). For this reason, 2-butanone was termed a putative carbon dioxide mimic. In a field study the compound was shown to be an effective replacement of carbon dioxide in attracting the malaria vector *An. funestus* (Mweresa et al., 2014; Mburu et al., 2017). Large scale mass trapping of malaria vectors using odour-baited traps supplemented with 2-butanone led to a reduction of over 69% *Anopheles funestus* population and a reduction of over 30% malaria prevalence (Hiscox *et al.*, 2016; Homan *et al.*, 2016).

As an alternative to the use of odour baits in the pull system, traps that utilize other host-seeking cues or attractive cues other than olfactory ones could be explored which can be used to catch mosquitoes that have been diverted by spatial repellents (Joshi *et al.*, 1975; Mathenge *et al.*, 2005; Murchie *et al.*, 2016). The use of non-odour traps may be necessary if the repellent insecticide affects odorant receptors leading to a reduction in perception of host odours (Ditzen *et al.*, 2008; Murphy *et al.*, 2013; Tsitoura *et al.*, 2015; Ponlawat *et al.*, 2017; Tzotzos *et al.*, 2018). Tapping into the mosquito visual cues for example, may be explored as an option to ensure that in case mosquitoes odorant receptors are compromised following exposure to repellents, the mosquitoes would still be able to respond to light cues (Murchie *et al.*, 2016; LeClair *et al.*, 2017; Ponlawat *et al.*, 2017).

Push-pull strategies have been used to control disease-transmitting insects of both agricultural and medical importance by using repellents identified among host odours combined with killing agents (Cook *et al.*, 2007; Takken, 2010; Wachira B.M., 2016). In principle the strategy may have the potential to control outdoor transmission of malaria in many communities, since host-seeking mosquitoes that are repelled from their intended hosts will be eventually lured towards specific lethal sites, therefore reducing chances of transmitting diseases through reduced host-vector contact and population suppression (Cook *et al.*, 2007). Laboratory and semi-field studies in Germany that targeted *Aedes* mosquitoes showed a reduction of up to 50% of bites on human volunteers when a spatial repellent (catnip oil) was used in combination with BG-sentinel traps (Obermayr *et al.*, 2015).

Another study targeting malaria vectors in central America showed a reduction in house entry behaviour of *An. vestitipennis* when transfluthrin was applied indoors, with a simultaneous increase in mosquito catches in the outdoor traps (Wagman et al., 2015b). Other studies done in Kenya showed reduction in house-entry behaviour of malaria vectors of between 61% and 99% and increased mosquito capture in the outdoor baited traps when several spatial repellents were applied indoors when house entry behaviour was monitored overnight (Menger et al., 2015; Menger et al., 2016).

More knowledge is required for the development of a 'push-pull' product for large scale roll out specifically targeting outdoor-biting mosquitoes. The product should target the mosquito's response to human odours through the release of both spatial repellents and synthetic lures mimicking human scent released from traps (Dia *et al.*, 2005; Qiu *et al.*, 2010). The ideal mosquito response to the push-pull strategy would be initial diversion from the protected human but a continued host-seeking behaviour to allow for the detection of the synthetic lures once they are in proximity to the odour plumes emitted by traps or other lure-and-kill devices. Quantification of the chemicals present in the air surrounding the system will give an indication of the amount of active ingredients that mosquitoes are exposed to, while also providing data for health and environmental safety assessment of the system (Martin *et al.*, 2013; Hogarh *et al.*, 2018; Kwan *et al.*, 2018). Understanding the basis of the decision made by mosquitoes on how to proceed following exposure to this cocktail of cues is important in determining the effectiveness of the proposed push-pull tool in controlling outdoor mosquitoes (Cardé *et al.*, 2010b; Suh *et al.*, 2014).

My study seeks to characterize the mosquito response to human hosts in the presence of spatial repellents and synthetic odours while determining effective concentrations required to reduce human bites by host-seeking anopheline mosquitoes. Additionally, the dissemination of repellents using fabrics such as eave wraps will be explored in comparison to eave screening. The potential of 2-butanone as a carbon dioxide replacement will be explored in the pull system, and light traps explored as possible alternatives in reducing the population of diverted mosquitoes. The findings aim to further develop the application of the push-pull strategy for anopheline mosquito vector control.

This thesis:

This thesis describes the host-seeking behaviour of *Anopheles* mosquitoes in the presence of both spatial repellents and synthetic lures and seeks to define conditions that are necessary to achieve protection to susceptible humans outdoors. Data generated on the selection of the constituents of the push-pull system through systematic examination of various candidate components offers information on the process behind this control tool to define parameters within which protection is conferred to humans outdoors and indoors. Mosquito behavioural responses to human odours compared to light cues are studied in the presence of transfluthrin as a guide to optimise tools needed to work in synergy to provide maximum host protection.

The work described in this thesis is based on the following specific objectives:

1. Develop a push-pull system that is protective against anopheline mosquitoes to humans outdoors under semi-field conditions
    a. Systematically test spatial repellents and odour-baited traps and determine the combination that offers the best protection to humans outdoors.
    b. Quantify the chemical components of the push-pull system in air samples.
2. Evaluate the efficacy of 2-butanone as a replacement for CO_2 in odour-baited traps designed to attract host-seeking *Anopheles arabiensis* mosquitoes.
3. Determine response of host-seeking *Anopheles* mosquitoes to synthetic lures in comparison to UV light traps both in the absence and the presence of transfluthrin.
4. Investigate the impact of transfluthrin-treated fabric used as an ‘eave wrap’ on outdoor and indoor human biting rates compared to an ‘eave screen’
5. Determine effect of the push-pull system on indoor and outdoor-biting natural mosquitoes in comparison to transfluthrin only (push) and an odour-baited trap only (pull).

Chapter 2 provides a description of the development of the push-pull system and provides data that show when outdoor-protection was achieved. Details are provided on the various repellents that were tested for consideration as the push component and how they compared to each other, while the pull component was tested on its ability to provide protection to humans from outdoor-biting mosquitoes. The best performing push and pull systems were combined and the sum of their protection on humans against outdoor-biting mosquitoes quantified. The concentration of the constituent chemicals in the air was measured at different heights and distances from point of release to describe spatial variation important to explain mosquito behaviour.

Chapter 3 provides empirical evidence of changes in preference of host-seeking mosquitoes to either odour-baited traps or CDC UV light traps when transfluthrin is present in relatively close proximity compared to when it is absent, and attempts to inform future developments of the push-pull set up where both the push and the pull components are set up in close proximity.

Chapter 4 compares the protection of transfluthrin-treated eave wraps on both outdoor and indoor biting mosquitoes to that conferred by eave screens. Additional comparisons are made to their untreated counterparts as a measure of any inferiority in protection between the two and to define the scope of protection provided by eave wraps compared to eave screens.

Chapter 5 describes afield assessment of the push-pull system by providing data on the response of natural mosquitoes and protection conferred both indoors and outdoors by the push-pull system. The data also allow comparisons for degree of protection between the repellent eave wrap applied alone compared to the odour-baited trap applied alone to assess if synergistic protection in the push-pull system occurred.

Chapter 6 provides a summary and discussion of findings that aims to guide the future of push-pull mosquito control in Kenya.

Chapter 2

Evaluating putative repellent 'push' and attractive 'pull' components for manipulating the odour-orientation of host-seeking malaria vectors in the peri-domestic space

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Chapter 2

Abstract

Background Novel malaria vector control approaches aim to combine tools to work in synergy for maximum protection. This study aimed to evaluate novel and re-evaluate existing, putative repellent ‘push’ and attractive ‘pull’ components for manipulating the odour-orientation of malaria vectors in the peri-domestic space.

Methods *Anopheles arabiensis* outdoor human landing catches and trap comparisons were implemented in large semi-field systems to (1) test the efficacy of Citriodiol® or transfluthrin-treated fabric strips positioned in house eave gaps as push components for preventing bites; (2) understand the efficacy of an MB5-baited Suna-trap in attracting vectors in the presence of a human being; (3) assess 2-butanone as a CO_2 replacement for trapping; and (4) determine the protection provided by a full push-pull set up. The air-concentrations of the chemical constituents of the push-pull mosquito control tool were quantified.

Results Microencapsulated Citriodiol® eave strips did not provide any outdoor protection against host-seeking *An. arabiensis*. Transfluthrin-treated strips significantly reduced the odds of a mosquito landing on the human volunteer (OR 0.17; 95% CI 0.12-0.23). This impact was lower (OR 0.59; 95% CI 0.52-0.66) during the push-pull experiment which was associated with low night-time temperatures likely affecting the transfluthrin vaporisation. The MB5-baited Suna trap supplemented with CO_2 attracted only a third of the released mosquitoes in the absence of a human being, however, with a human volunteer in the same system, the trap caught less than 1% of all released mosquitoes. The volunteer consistently attracted over two-thirds of all mosquitoes released. This was the case in the absence (‘pull’ only) and in the presence of a spatial repellent (‘push-pull’), indicating that in its current configuration the tested ‘pull’ does not provide a valuable addition to a spatial repellent. The chemical 2-butanone was ineffective in replacing CO_2. Transfluthrin was detectable in the air space but with a strong linear reduction in concentrations over 5 metres from release. The MB5 constituent chemicals were only irregularly detected, potentially suggesting insufficient release and concentration in the air for attraction.

Conclusion This step-by-step evaluation of the selected ‘push’ and ‘pull’ components led to a better understanding of their ability to affect host-seeking behaviours of the malaria vector *Anopheles arabiensis* in the peri-domestic space and helps to gauge the impact such tools would have when used in the field for monitoring or control.

Keywords: Malaria, Vector control, Outdoor-biting, Spatial repellent, PMD, Citriodiol®, Transfluthrin, GC-FID, semi-field study

Chapter 2

Background

In spite of the impressive efforts made in the past two decades, progress in the fight against malaria has stagnated in recent years(Bhatt *et al.*, 2015a; Bhatt *et al.*, 2015b; WHO, 2019).A large proportion of the reduction in malaria has been attributed to vector control, yet research and operational practice have concentrated on the development of chemotherapy and vaccines, with vector control not expanding its arsenal beyond long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS)(Hemingway *et al.*, 2016). Increased pyrethroid resistance in malaria vectors(Churcher *et al.*, 2016; Ranson *et al.*, 2016), shifts in mosquito biting behaviour from predominately endophagic to more exophagic populations (Meyers *et al.*, 2016; Limwagu *et al.*, 2019) and earlier biting (Mathania *et al.*, 2016) demand the re-evaluation of contemporary practices and the development of additional tools addressing current limitations. The World Health Organization (WHO) endorsed the universal use and application of LLINs and IRS as tools in the fight against malaria(WHO, 2014). Both of these tools primarily target indoor-biting mosquitoes which contribute to almost 80% of all malaria transmission (Saavedra *et al.*, 2019). Whilst the remaining outdoor transmission increases in importance once the indoor tools are effectively applied(Killeen *et al.*, 2012; Killeen, 2014), no outdoor tools have been approved by WHO for supplementary mass application(WHO, 2019).

The use of spatial repellents has been proposed to provide protection against bites at a distance from the point of application which could not only provide potential protection to multiple persons but may also lead to higher compliance due to reduced need for reapplication which is a barrier to effective use of tropical repellents (Grieco *et al.*, 2005; Achee *et al.*, 2012; Lynch *et al.*, 2016; Norris *et al.*, 2017).The ability to produce vector-free spaces would make spatial repellents ideal for application in the peri-domestic space, defined as in-and around the outside of the house(Achee *et al.*, 2012). Several insecticides already used in public health have, to varying degrees, spatial repellent effects on various mosquito species (Bibbs *et al.*, 2018). These insecticides volatilize more readily than other adulticides and repel, even in instances when the vectors are intrinsically resistant to pyrethroids (Bowman *et al.*, 2018; Deletre *et al.*, 2019). One pyrethroid that exhibits spatial repellent properties against mosquitoes at sub-lethal concentrations is transfluthrin (Ogoma *et al.*, 2012b; Bibbs *et al.*, 2018; Martin *et al.*, 2020). However, in the light of growing pyrethroid resistance it would also be desirable to search for novel active compounds. For example, Citriodiol® sourced from *Eucalyptus citriodora* oil, which includes a minimum 64% para menthane-3, 8-diol (PMD) as the active ingredient, is used in topical skin repellents (Barasa *et al.*, 2002; Carroll *et al.*, 2006; Carroll *et al.*, 2019)and has been suggested to have spatial repellent properties(Menger *et al.*, 2014).

There is a possibility that, when used on their own, spatial repellents might lead to increased biting on unprotected persons through diversion of host-seeking vectors from treated to untreated spaces(Maia *et al.*, 2016). To prevent diverted vectors from finding alternative hosts, supplementary tools such as odour-baited traps might be combined with spatial repellents. Odour-baited mass trapping, as a single tool, has shown to reduce *An. funestus* densities indoors in a recent field trial (Homan *et al.*, 2016). Spatial repellents and odour-baited traps target opposing odour-mediated orientations of the mosquito and therefore may work synergistically in a 'push-pull' system (Takken, 2010; Menger *et al.*, 2014; Menger *et al.*, 2015; Menger *et al.*, 2016).

The term 'push-pull' was first conceived as a strategy for insect pest management in Australia in 1987(Pyke *et al.*, 1987)and the concept is now frequently applied in the control of agricultural pests(Cook *et al.*, 2007; Yan *et al.*, 2015). The intervention not only offers repulsion from the intended host, but rather redirects them to an alternative that does not lead to disease(Cook *et al.*, 2007; Takken, 2010; Njihia *et al.*, 2014; Wagman *et al.*, 2015b).An adaptation of this tool for vector control was developed to curb transmission of trypanosomiasis. Cattle provided with a repellent worn on the neck as a push, were supplemented with insecticide-treated targets which acted as attractive pull components that killed the flies that landed on them(Saini *et al.*, 2017).The reduction in tsetse fly populations was more strongly associated with a combined push-pull set up than with the repellent and attractant when used separately or not at all(Saini *et al.*, 2017).To develop such a 'push-pull' strategy for malaria vector control, it is necessary to determine the efficacy of the potential components individually and in combination to understand their contribution to protecting human hosts from bites. The push-pull strategy for malaria vector control targets the odour-mediated orientation of female mosquitoes when searching for a human host and aims to manipulate this behaviour. This requires that effective quantities of the repellent and odour attractants are perceived by the targeted mosquito species within the space that should be protected(Martin *et al.*, 2013). Quantification of the airborne concentrations of the chemical constituents of the push-pull control tool might help interpret behavioural responses recorded in bioassays and gauge the influence of weather conditions (Ramesh *et al.*, 2001; Martin *et al.*, 2013; Kwan *et al.*, 2018). Such information might inform the spatial arrangement of the push-pull system and assist in identifying needs for improvements of release rates of individual components. Importantly, quantification of chemicals in the air allows for monitoring of safe levels, especially amounts inhaled by humans or levels available to susceptible non-target hosts (Ramesh *et al.*, 2001; Nazimek *et al.*, 2011).

This study aimed to evaluate novel and re-evaluate existing, putative repellent 'push' and attractive 'pull' components for manipulating the odour-orientation of malaria vectors in the peri-domestic space with the aim to develop a 'push-pull' system that reduces bites and kills vectors. Five objectives were pursued: (1)

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To test the efficacy of fabric strips treated with either microencapsulated Citriodiol® or with an emulsified concentrate of transfluthrin positioned in open eave gaps on houses as a push component for preventing *Anopheles arabiensis* bites outdoors; (2) To understand the efficacy of an MB5-blend baited Suna-trap in attracting (pulling) *An. arabiensis* to the trap in the presence of a human being; (3) To assess the possibility of replacing CO_2 produced from yeast-sugar fermentation with the putative CO_2 replacement, 2-butanone, in the Suna trap; (4) To determine the degree of protection for a human host against mosquito bites by combining push and pull components; and (5) To quantify the air concentrations of the chemical constituents of the push-pull mosquito control tool.

Methods

Study site

All experiments were carried out in semi-field systems made up of four netting-screened green-houses located at the International Centre of Insect Physiology and Ecology's Thomas Odhiambo Campus (*icipe*-TOC) at Mbita, in Homabay County, western Kenya (0°26'06.19"S, 34°12'53.13"E; altitude 1,137 m). The majority of experiments (Table 1) were carried out in two large semi-field systems (Amiran Ltd, Nairobi, Kenya) measuring 27 m in length, 11 m in width and 4.3 m at the highest midpoint (Figure 1).

The two large systems were located in parallel, 10 m apart from each other. The roof covers were made from translucent water-proof Solarig™ material (Amiran Kenya Ltd.) and the sides were made of a 17-mesh netting material (17 apertures per every linear inch of mesh). One wooden make-shift hut made from plywood walls attached to angle irons, with grass thatch applied on an open gable roof, was included in each system at opposite ends approximately 5 m away from the shorter walls.

The huts were 6.5 m long and 3.5 m wide with a maximum height of 2.5 m (Figure 1). Between the roof and the walls was a 0.1 m eave gap; a size that was representative of the open eave gaps typical in traditional western Kenyan houses and in other rural African areas (Njie *et al.*, 2009; Wanzirah *et al.*, 2015; Jatta *et al.*, 2018a).The doors and windows of the experimental huts were fully mesh-screened. Mosquitoes could only enter and exit the huts through the eave gaps during experiments.

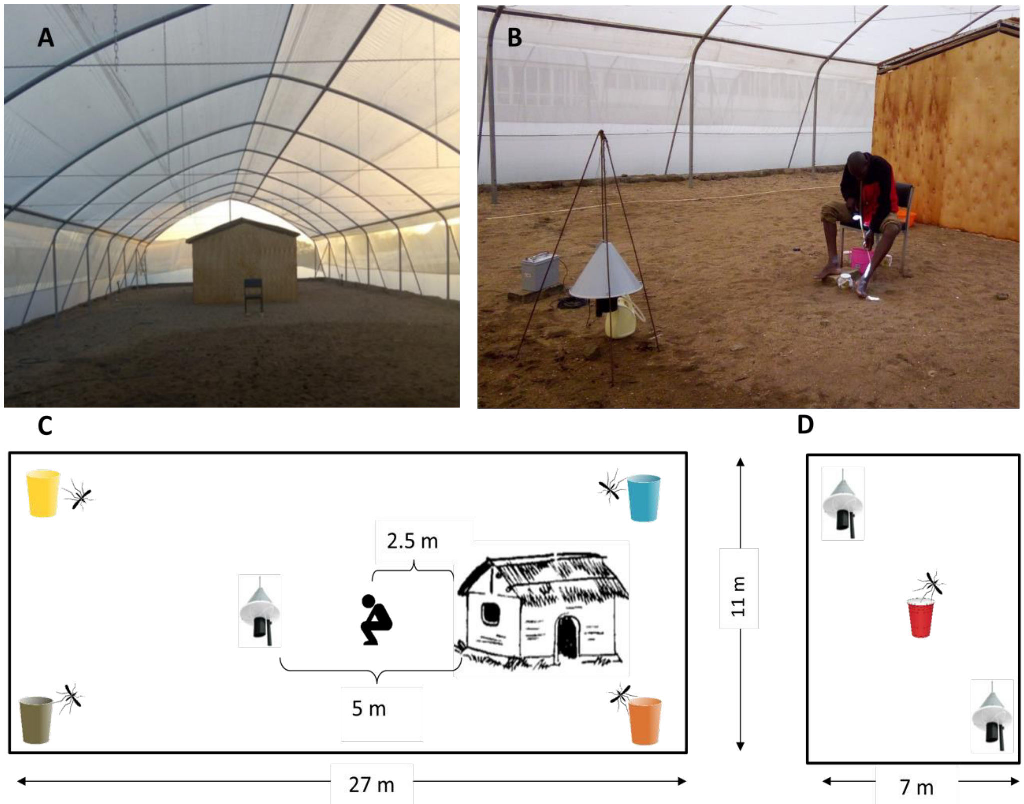

Figure 1. Pictorial presentation of the experimental set ups in the semi-field systems. A. View into the large tunnel-shaped semi-field system; 11 m wide and 27 m long. **B.** Volunteer implementing human landing collections between the experimental hut and the Suna trap in the larger system. **C.** Schematic description of experiments including HLC outside the hut 2.5 m away from the hut (eave treatments) and the Suna trap. Colour-coded mosquitoes were released from all four corners of the system. **D.** Schematic description of experiments in the small semi-field system, 11 m long and 7 m wide, where different trap configurations were tested with two traps included in the system.

Table 1: Summary of the experiments in relation to research questions.

	TEST Treatment	CONTROL Treatment	Human landing catch	Test & control independent or in competition*
Experiment 1				
	• What is the human biting rate of *Anopheles arabiensis* released in the semi-field systems between 19.00 and 23.00 h in the absence of any treatment? • Are the two semi-field systems comparable in the results they generate? • Are there any differences in catching efficiency/attractiveness of HLC volunteers?			
1.1	no treatment	no treatment	yes	independent
Experiment 2				
	• Can Citriodiol® and/or transfluthrin-treated strips located at eave gaps reduce *An. arabiensis* biting rates compared to untreated controls?			
2.1	1 g/m^2 microencapsulated Citriodiol®	untreated cotton fabric	yes	independent
2.2	11g/m^2 microencapsulated Citriodiol®	untreated cotton fabric	yes	independent
2.3	1.25g/m^2 transfluthrin fabric	untreated hessian fabric	yes	independent
2.4	2.5g/m^2 transfluthrin fabric	untreated hessian fabric	yes	independent
Experiment 3				
	• How effective is the MB5 baited Suna trap in attracting insectary-reared *An. arabiensis* in a large semi-field system in the absence and presence of a human being? • How does the MB5 cartridge perform in comparison to nylon strips impregnated by investigator? • Does 2-butanone combined with MB5 perform equally well in attracting insectary-reared *An. arabiensis* in a large semi-field system as CO_2 produced from molasses fermentation? • How does the trapping efficacy compare between Suna traps baited with CO_2only and Suna traps baited with the synthetic MB5 lure in addition to CO_2?			
3.1	MB5-**cartridge** baited Suna trap supplemented with CO_2	MB5-**nylon strip** baited Suna trap supplemented with CO_2	no	competing
3.2	MB5-cartridge baited Suna trap **with 2-butanone**	MB5-cartridge baited Suna trap **with CO_2**	no	competing
3.3	MB5-cartridge baited Suna trap **with 2-butanone**	unbaited Suna trap (no MB5, no CO_2, **only suction fan**)	no	competing
3.4	**MB5-**cartridge baited Suna trap **with CO_2**	unbaited Suna trap (no MB5) supplemented **withCO_2 only**	no	competing
3.5	MB5-cartridge baited Suna trap supplemented **with CO_2**	unbaited Suna trap (no MB5, no CO_2, **only suction fan**)	yes	independent
3.6	MB5-cartridge baited Suna trap **with2-butanone**	unbaited Suna trap (no MB5, no CO_2, **only suction fan**)	yes	independent
Experiment 4				
	• What is the impact of a complete push-pull set-up on the *An. arabiensis* biting rate?			
4.1	transfluthrin 2.5g/m^2 eave wrap + MB5-cartridge & with CO_2	untreated eave wrap + unbaited Suna trap	yes	independent

*Two semi-field systems were used for testing test and control treatments independently but concurrently. The treatments were randomly allocated to the two systems. Competing tests were set in the same semi-field system.

Few experiments (Table 1) were done in smaller-sized semi-field systems measuring 11 m in length and 7 m in width, with 2.5 m at the highest point (Figure 1). The walls of these were screened with fibreglass netting of the same mesh size as the large systems while the roofs were made of translucent polycarbonate (Okal *et al.*, 2015). Ambient temperatures inside the semi-field systems ranged between a minimum of 18°C at night and maximum of 50°C during the day as monitored with data loggers suspended in the middle of the semi-field systems. During the nightly experiments between 19.00 and 23.00 h the average temperature ranged between 21°C and 24°C. The natural floor in all four semi-field systems was covered with a layer of around 20 cm of sand and was watered daily for two hours, prior to the experiments with free-flying mosquitoes, to maintain a relative humidity in the systems of around 70%. A summary of all experiments is found in Table 1. All experiments with human landing volunteers included were implemented in the large semi-field systems.

Mosquitoes

All experiments were implemented with host-seeking females of *An. arabiensis* Mbita strain, aged between 3 to 5 days post-emergence. Mosquitoes were reared under ambient conditions at *icipe*-TOC following standard operating procedures(Mbare *et al.*, 2013). Nulliparous mosquitoes that had not taken a blood-meal previously were activated to host-seek by placing a human hand near the outside of the mosquito cage and only those that responded to human odours were aspirated and used in experiments. In experiments including ahumanvolunteer,160 females were released in each semi-field system per experimental night. In experiments including traps only, 200 females were released in each system per night. The mosquitoes were transferred from rearing cages into release cups using mouth aspirators. In the release cups they were starved from water and glucose for a minimum of three and a maximum of five hours prior to release. Anticipating that the orientation of a female mosquito in the system will be affected by the direction of air movement, obstructions like the hut and outside light sources, mosquitoes were released from cups in all four corners of a semi-field system to account for such factors. In experiments including a human, each of the four release cups contained40*An. arabiensis* females (total = 160 females). Mosquitoes in each release cup were dusted using a distinct colour of fluorescent dye to distinguish them according to the four corners of release (Verhulst *et al.*, 2013). In choice experiments with traps only, the traps were rotated through the corners of the semi-field system and the mosquitoes released from one release cup in the centre of the screen house.

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Repellent-treated fabrics (push component)

A passive release mechanism for spatial repellents was favoured in this project in order to reduce the operational complexity that would come with an electricity-powered active dispenser. Hence, the two test compounds, Citriodiol® and transfluthrin, were both presented on fabrics which can be easily attached to open eave gaps on houses(Menger *et al.*, 2016).

Citriodiol® (Citrefine International Ltd)was microencapsulated by Devan Chemicals, Portugal and applied to fabric by Utexbel, Belgium using the solvent evaporation technique with poly lactic acid as a shell material as previously described (Liu *et al.*, 2005; Menger *et al.*, 2015). The fabric was shipped to Kenya and stored in a cold and dark room prior to use. Two fabric weights with two loads of Citriodiol® were tested. The first was a100% cotton fabric(65g/m^2)with1g/m^2Citriodiol®and the second had a fabric weight of 550g/m^2 with a Citriodiol® load of 11g/m^2(microcapsules for both were 15 micro-m with 17% wt. of the active ingredient of para menthane-3, 8-diol; PMD).

Transfluthrin(Bayer Global, Leverkusen, Germany) was obtained as emulsified concentrate(EC) of 0.2g/ml and applied on hessian fabric (obtained as burlap material from local markets in Kenya) to achieve two final loads on the fabric; namely 1.25g/m^2 and 2.5g/m^2(Ogoma *et al.*, 2017).The impregnation of the hessian fabric was done in the laboratory at *icipe*-TOC where the respective amount of transfluthrin EC was added into to water that was sufficient for wetting the entire length of fabric without any water remaining. The fabric was soaked well and dried in the shade overnight then wrapped up in aluminium foil and stored in a cold (4°C) and dark room prior to use.

The treated fabrics were cut into strips measuring a length of 21m, corresponding to the perimeter of the eave gaps of the experimental huts and a width of 0.05m, corresponding to half of the width of the eave gap. Correspondingly, untreated fabric strips were prepared in the same dimensions and used for the control experiments. The fabric strips were fixed half an hour prior to mosquito release with flexible aluminium wires in such a way that they were covering only part of the eave gap leaving a similar space above and below(2.5cm each) to allow for movement of air. They represented an incomplete, easy to fix fabric strip along the gaps, not an eave screen. The fabric strips were removed in the morning and stored in the cold room till the next experimental night. Fabric strips were used continuously for a maximum of eight experimental nights. Experiments were done for 16 nights; hence two strips were used per experiment.

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Suna trap and odour lure (pull component)

Odour-baited Suna traps were used throughout as pull devices. The trap's development, appearance and operation is described in detail elsewhere(Hiscox *et al.*, 2014).The principle odour bait re-evaluated in experiments was a synthetic chemical blend aiming to mimic human host odours and has previously been published under the name 'Mbita Blend 5' or MB5 (Mukabana *et al.*, 2012a; Mweresa *et al.*, 2014). The MB5 comprised of ammonia (2.5% in water), L-(+)-lactic acid (85% in water), tetradecanoic acid (0.00025g/l in ethanol), 3-methyl-1-butanol (0.000001% in water), and butan-1-amine, prepared at a concentration of 0.001% in paraffin oil(Menger *et al.*, 2014)and was recently associated with significant reductions in *An. funestus* populations during a mass-trapping vector control trial (Homan *et al.*, 2016).Two dispensing substrates of MB5 were compared. As in previously published work, MB5 was presented on nylon strips(Okumu *et al.*, 2010a; Mweresa *et al.*, 2015)where each strip was treated with one chemical of the blend and consequently five strips inserted in the trap. This was compared to a novel, slow-release cartridge developed by Biogents (Biogents Cartridge Lure (Mosquito Attractant) - LI-MR-43, Regensburg, Germany) containing the same five chemicals.

Carbon dioxide has been repeatedly reported as essential in combination with an odour blend for attracting host-seeking malaria vectors (Takken *et al.*, 1989; Healy *et al.*, 1995; Gillies, 2009) and remains one of the most challenging obstacles to area-wide operational use of odour-baited traps. Carbon dioxide gas released from cylinders is not manageable under field conditions; hence a previously developed method of producing CO_2 from fermenting sugar or molasses solution using yeast is now widely used (Smallegange *et al.*, 2010; Harwood *et al.*, 2014; Sukumaran *et al.*, 2016). However, the amount of sugar or molasses needed for every trap night is still prohibitive for operational vector control. The chemical 2-butanone has been proposed as a CO_2 mimic for mosquitoes, but the literature is controversial (Turner *et al.*, 2011; Mburu *et al.*, 2017). Here, CO_2 from fermentation was compared with 2-butanone treated (0.1ml) nylon strips (Mburu *et al.*, 2017) to gain a better understanding of its effectiveness as a supplement of the odour-bait in a Suna trap for reducing *An. arabiensis* bites.

In experiments including a human volunteer, a single odour-baited Suna trap was positioned 5 m from the experimental hut; with the volunteer seated mid-way between the hut and the trap in a straight transect (Figure 1C). The trap was suspended above the ground using a tripod (Hiscox *et al.*, 2014; Mburu *et al.*, 2017)with the main odour-release point, which is the bottom of the funnel, approximately 0.3 m off the ground. In experiments without a human volunteer, two traps were positioned at diagonally opposite corners of the small semi-field system approximately 13m from each other and less than 1m from the walls of the system (Figure 1D).

Estimation of vector landing rates

Human landing catches (HLC) were carried out as the primary outcome measurement and were conducted on a randomly rotating basis by four adult men (aged between 18 and 50 years) seated 2.5m away from the experimental hut to mimic outdoor biting in a natural setting where people would spend time outside the house during the evening hours. Two volunteers were required per night. In preparation of the experiments, they cleaned their feet and lower legs with odourless soap and took position on a chair as shown in Figure 1. Collections were done for four hours from 19.00h to 23.00h, with volunteers mouth-aspirating host-seeking *An. arabiensis* females as soon as they landed on their lower legs(Kenea *et al.*, 2017). The mosquitoes were transferred to collection cups, separated hourly. Protective jackets and shoes were worn to protect heads, arms and feet against bites and torches were used for visualization of mosquitoes when aspirating. Volunteers were randomized to the semi-field system and to the experiment to avoid any bias due to differences in collection efficacy and individual attractiveness to mosquitoes.

Experimental procedures

All experiments and their guiding research questions are detailed in Table 1. Those including human landing catches were conducted as set in two semi-field systems concurrently. All experiments were replicated over 16 nights. A baseline experiment was conducted to understand the mosquito response rate to human volunteers in the two semi-field systems in the absence of any behaviour modulating chemicals. This provided a reference for other experimental sets and helped gauge any differences in attractiveness and catching efficiency of the volunteers or between the two semi-field systems. This experiment also helped to understand the response rates that can be expected from receptive host-seeking mosquitoes in the system. Following this, a threshold was established where, if the response rate in the presence of a human volunteer was below 50% in the control treatment, results were discarded, and the replicate repeated. Spatial repellent treatments were rotated weekly given the need to air between treatments to avoid cross-contamination. Experiments were done for four consecutive nights then all test devices and chemical odours were withdrawn from the semi-field systems for three days. In the following week, the treatments were crossed over between the semi-field systems. Trap only experiments were conducted through the night from 19.00h to 07.00h the next morning.

Simulation-based power analysis

A simulation-based power analysis (Johnson *et al.*, 2015)was implemented for a 2x2 Latin square experiment with two treatments each tested by four volunteers in two semi-field systems. The aim was to be able to measure a 50% reduction in human landing rate; hence a recapture rate of 60% in the control and 30% in the push-pull experiment was used for the estimation. Assuming160 mosquitoes released in each semi-field system, and assuming10% dispersion due to variability between the semi-field systems, 10% variations between mosquito releases, and 50% variability between the HLC volunteers, 1000 simulations resulted in an estimated power of 0.94 (95% CI 0.87 - 0.98) to detect a 50% reduction in human landing rate for 16 replications.

Air sampling and detection of volatile chemicals released by the push-pull components

Air was sampled in one of the large semi-field systems in the presence of a fully set push-pull system, consisting of 2.5g/m^{2}transfluthrinfabric strips on eave gaps and a Suna trap baited with MB5nylon strips and CO_2 generated through fermentation of molasses. Air was pumped through adsorbent Texan traps (30 mg; GERSTEL-Twister Desorption glass liners from GERSTEL, Muelheim an der Ruhr, Germany, glass wool from Supelco, Bellefonte, PA, USA and 25 mg of Tenax® TA polymer 60–80 mesh from Supelco, Bellefonte, PA, USA).Micro-diaphragm gas pumps were used at the rate of 400ml/min resulting in a total of 120 litres of air passing through each trap over a five hour sampling period (18.00-23.00 h),chosen to align with the time period when human landing catches were implemented under experimental conditions. The air sampling was carried out in the absence of a human to focus on the chemicals released by the push-pull components. All chemicals collected were reported as concentrations averaged over the time-period of trapping and calculated as nanograms per litre of air sampled; subsequently referred to only as 'concentration' in ng/l.

Twelve locations were sampled in a transect between the transfluthrin-treated fabric at the experimental hut and the odour-baited Suna trap placed at a distance of 5 m away from the hut (Figure 2).
Sampling was done every 1 m between the fabric (house wall) and the trap, at four distances. At every distance, sampling was done at three heights: 0.5 m, 1.0 m and 1.5 m (Figure 2).Sampling was replicated over 5 non-consecutive days, with each set up using freshly treated eave fabric and new nylon strips for the odour-blend to ensure consistency in the initial concentrations. At the conclusion of each sampling event, adsorbent filters were stored at -80°C degrees until chemical analysis. For quantification, trapped volatiles were eluted using dichloromethane (DCM; CAS 75-09-2, Merck, Massachusetts, USA) and

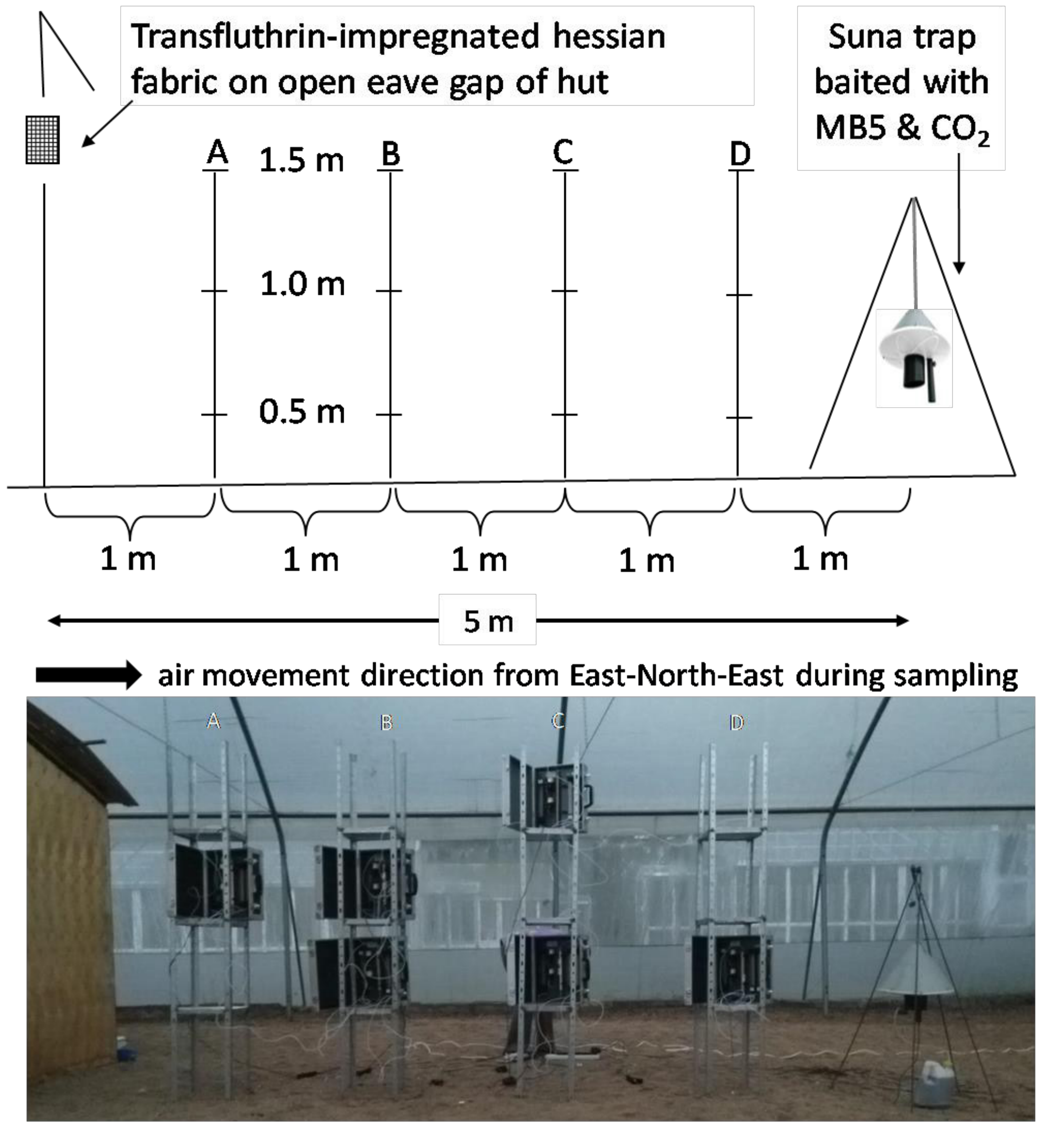

Figure 2. Pictorial presentation of air sampling set up. Air-entrainment pumps were positioned at 1 m (A), 2 m (B), 3 m (C) and 4 m (D) distance from the experimental hut where a transfluthrin-treated fabric was positioned at the eave gap. The baited Suna trap was 5 m away from the hut. Sampling was done at every position at 3 heights: 0.5 m, 1.0 m, and 1.5 m above the ground.

analysed using gas chromatography (GC) with flame-ionization detection (GC-FID; Agilent 7890B, Agilent Technologies, California, USA; (Buse *et al.*, 2019)).
The lowest detection temperature was set at35 °C and the highest was set at 280°C. A Solgewax (SGE, Australia) column, 30m long and 0.25mm in diameter with an internal diameter of 0.2µm, was used.
To obtain calibration lines for quantification of air concentrations for all the push-pull constituent chemicals (except L-lactic acid and ammonia solution) concentration gradients were obtained by preparing dilutions of the chemicals from the stock solutions ranging from 0.5 to 10ng/µl resulting in the preparation of the following concentrations: 0.5µg/µl, 1.0 µg/µl, 2.0 µg/µl, 4.0 µg/µl, 6 µg/µl, 8 µg/µl and 10 µg/µl. Each concentration was injected separately into the GC-FID, then the area under the curve determined and plotted against the concentration. A linear equation was obtained by plotting all the concentrations against all the areas of each chemical where y represented the area under the curve while x represented the concentration in nanograms per litre of air. All linear equations met the minimum qualification of R^2 value of 0.98. Subsequent determination of concentration was determined by obtaining the area under the curve directly from the GC-FID and solving for x in the linear equation of each chemical(Oliveira *et al.*, 2010; Guo *et al.*, 2016; Feng *et al.*, 2018).
To determine direction and strength of air movements as well as temperature during collections, a long-range wireless wind logger (Navis WL 11X, NAVIS Elektronika, Kamnik, Slovenia) was set up at 2 m height next to the Suna trap during the air sampling period. Logging of parameters was done in five-minute intervals.

Data analysis

Analyses of experimental data were done using R version 3.5.1(R Core Team, 2018). Data were descriptively explored and presented by generating box plots, where the boundary of the box closest to zero indicates the 25thpercentile, the black line within the box marks the median, and the boundary of the box farthest from zero indicates the 75thpercentile. Whiskers above and below the box indicate the 10thand 90thpercentiles. All mosquito catches were analysed as proportions(number of mosquitoes attempting to bite either out of the total number released in the system or out of the total number recollected with HLCs and/or traps) using generalized linear mixed models with the experimental night and HLC volunteer(where applicable) included as random factors. All proportions were modelled using binomial probability distributions with logit link functions fitted. Treatment group was included as the fixed factor in the models with the control group as reference. The semi-field system ID was also included as factor and retained in the final model only if significantly associated with the outcome. Where applicable, interactions were explored. All analyses of volatile chemicals in air samples were done by calculating the means and the standard error for measurements made at every position across the five sampling days. Analysis of variance to determine differences between sampling positions A-D and sampling heights 0.5-1.5m were done for each chemical detected.

Results

Experiment 1: Establishing landing rates of *An. arabiensis* in semi-field systems in the absence of treatments

On average, 67% (95% CI 62-72%) of the released mosquitoes were recaptured in semi-field system A and 62% (95% CI 56-67%) in system B within four hours of human landing collections (19.00-23.00 h) with volunteers seated 2.5 m away from the hut. Adjusting for time of collection and volunteer, the semi-field system (A or B) was not associated with the odds of recapturing a mosquito (OR 0.96, p=0.286; Table 2).

Table 2: **Association between outdoor *An. arabiensis* landing and time of collection, semi-field system, and volunteer.**

Explanatory variables in multivariable analysis	**Odds Ratio (OR)**	**Confidence Interval (CI)**		**p-value**
		Lower CI	**Higher CI**	
Collection time*				
19.00-20.00 h	1			
20.00-21.00 h	0.47	0.426	0.511	<0.001
21.00-22.00 h	0.28	0.250	0.307	<0.001
Semi-field system ID				
A	1			
B	0.96	0.868	1.043	0.286
HLC Volunteer ID				
no. 1	1			
no. 2	1.25	1.098	1.425	<0.001
no. 3	1.26	1.099	1.439	<0.001
no. 4	1.17	1.018	1.338	0.027

*no mosquitoes were captured between 22.00-23.00 h, hence this category not included in analysis

Collection time, however, was significantly associated with the outcome (Figure 3). The largest proportion of host-seeking mosquitoes was recaptured in the first hour, whilst none were captured in the 4th collection hour (22.00-23.00 h). The odds of collecting a landing mosquito decreased over time(Table 2, Figure 3).

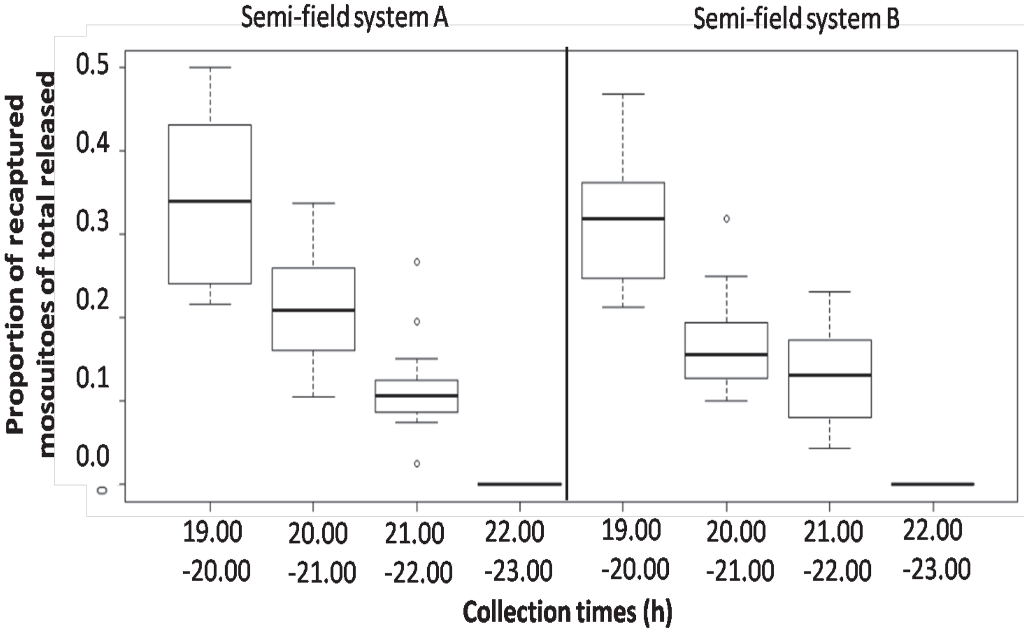

Figure 3. Hourly *Anopheles arabiensis* landing on human volunteer in two semi-field systems in the absence of any test. Based on human landing collections by four volunteers that were randomly rotated between the systems; out of all mosquitoes released (n=160/experimental night).

There was some variability in the collection efficiency between volunteers either due to attractiveness or skills, with one of the volunteers collecting fewer mosquitoes than the others (Table 2). Based on this, in consecutive analyses, the volunteer IDs were included as a random factor in the model. Host-seeking females were recaptured in similar proportions from all four release corners. There was no significant association between the human landing rate and temperature or relative humidity in the semi-field system during experimental nights.

These results, obtained in the absence of any treatment, confirmed that the insectary-reared *An. arabiensis* were highly responsive to a human blood host and that both semi-field systems supported reproducible results in the presence of an HLC volunteer. Subsequently, this set of experiments served as a reference for all following experiments with various treatments included.

Experiment 2: Investigating potential push components for a push-pull vector control strategy

Microencapsulated Citriodiol® fabric strips on open eave gaps

Neither the eave fabric encapsulated with 1 g/m^{2}Citriodiol®(p=0.488), nor the heavier fabric with 11g/m^{2}Citriodiol ® (p=0.633) were associated with a reduction in the proportion of mosquitoes landing on a volunteer when compared to untreated controls(Figure4A). In all experimental treatments, catches were similar and consistent, ranging between a median of 66-71% of all released mosquitoes recovered through HLC.

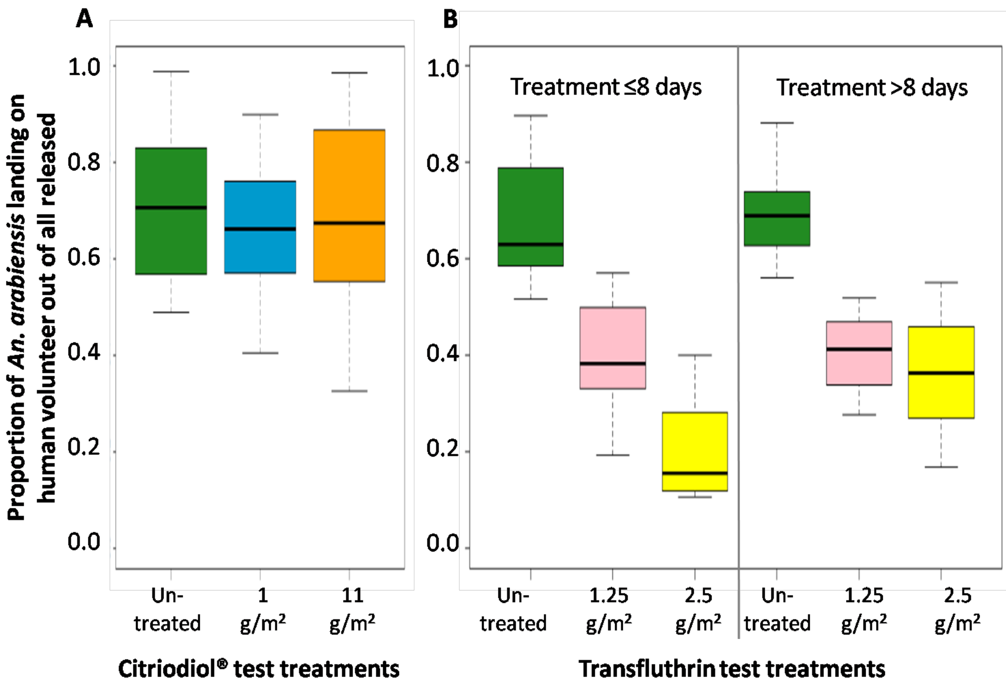

Figure 4 ***Anopheles arabiensis*** **host-seeking while exposed to putative spatial repellents in semi-field systems as estimated with HLC. (A)** Two formulations of microencapsulated Citriodiol(1g/m^2 and 11g/m^2) were tested in comparison to untreated control fabric. **(B)** Two impregnation concentrations of transfluthrin were tested on hessian fabric; 1.25g/m^2 and 2.5g/m^2, in comparison to untreated control fabric. The proportions are based on the total number of mosquitoes released (n=160/experimental night).

Transfluthrin EC impregnated hessian fabric strips on open eave gaps

Transfluthrin-treated fabric strips, at both treatment loads, were significantly associated with reduced human landing at a distance of 2.5 m away from the hut (Figure4B, Table 3). The odds of a mosquito landing on the volunteer in the presence of the 1.25 g/m^2 transfluthrin fabric was 2.5 times less (OR 0.39; Table 3) than the odds in the presence of the untreated control fabric. This was consistent over time, even when the fabric used in the experiment had been treated over eight days prior. The higher load of 2.5 g/m^2 resulted in a significantly higher protection with the odds of a mosquito landing 16 times less (OR 0.06; Table 3) than the odds of landing in the control. However, this protection reduced when the age of the treated fabric increased. When the fabric treatment had been done more than a week prior to testing, the odds of receiving a bite increased nearly 3-fold as compared to the fresh treatment (Table 3) but was still superior to the lower load. The median percentage of 63-70% of released mosquitoes landing on the HLC volunteer in the experiments with untreated fabric related well to the reference experiment without any treatments included and confirmed the reproducibility of the test system.

Table 3: Association between outdoor *An. arabiensis* landing and transfluthrin-treated fabrics (1.25 and 2.5 g/m^2) around eave gaps

Explanatory variables in multivariable analysis#	Odds Ratio (OR)	Confidence Interval (CI)		p-value
		Lower CI	Higher CI	
Transfluthrin concentration on fabric strip placed on open eave gap of hut				
Untreated	1			
1.25g/m^2	0.39	0.34	0.45	<0.001
2.5g/m^2	0.06	0.05	0.08	<0.001
Time post-treatment				
< 8 days	1			
>8 days	1.22	0.82	1.81	0.325
Interaction between transfluthrin concentration * time post-treatment				
1.25g/m^2 * <8 days	1			
2.5 g/m^2 * <8 days	1			
1.25g/m^2 * >8days	1.07	0.81	1.40	0.641
2.5 g/m^2 * >8days	2.78	2.02	3.82	<0.001

#Human landing collections were done nightly for 4 hours (19.00-23.00 h).

Experiment 3: Investigating pull components for a push-pull vector control strategy

Comparing the attractiveness of two odour dispensing substrates for use in Suna traps

Previously, the MB5 blend was prepared experimentally by manually treating nylon strips with the five chemicals (Okumu *et al.*, 2010a; Menger *et al.*, 2014; Mweresa *et al.*, 2015). For operational large-scale use, this would not be a feasible method, hence a commercial cartridge was developed (Biogents, Germany) which would be easy to use and replace by lay personnel. The competitiveness of the cartridge in attracting mosquitoes to the trap was tested by comparing it to a trap with treated nylon strips in the same small, semi-field system in the absence of a human volunteer.

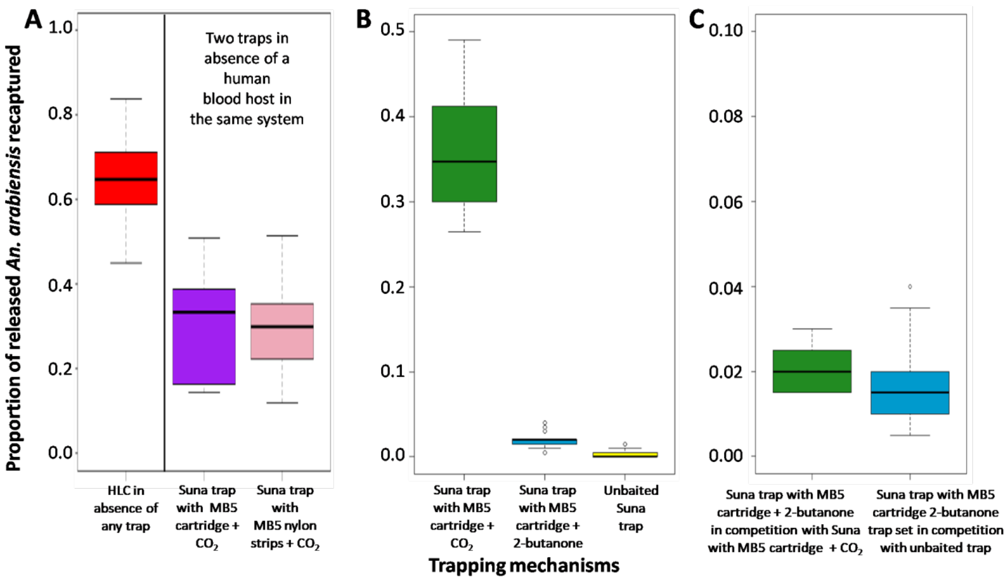

Figure 5. Exploring the impact of a novel MB5-release cartridge and 2-butanone instead of CO_2 on the *An. arabiensis* trapping efficiency of Suna traps under semi-field conditions. (A) Comparing the attractiveness of Suna traps baited either with MB5 treated nylon strips or MB5 containing cartridges (Biogents, Germany); both were supplemented with CO_2.The attractiveness of a human is shown as reference. **(B)** Evaluation of the effectiveness of 2-butanone as a CO_2 replacement for supplementation of MB5-baited Suna traps for attraction of *An. arabiensis*. **(C)** Box plot comparing the proportion of mosquitoes recaptured with 2-butanone supplemented traps when tested in choice tests. (Note the different scales of the Y-axes in the figures). A total of 200 host-seeking mosquitoes were released per experimental night. The traps were run over night for 12 hours.

In addition to the chemical blend, CO_2 was released in both traps during experiments using the fermentation method(Patrascu *et al.*, 2009; Mweresa *et al.*, 2014).

The CO_2-supplemented Suna traps were equally efficient in recapturing host-seeking *An. arabiensis* females released in semi-field systems, irrespective of the presentation of the chemical blend on nylon strips or enclosed in a slow-release cartridge. The two traps together recaptured 61% (95% CI 55-67%) of the released mosquitoes, with a balanced 1:1 distribution(approximately 30% in a single trap) between the two types of blend dispensers (Figure5A). Of all trapped females, 49% (95% CI 41-58%) were collected with the cartridge-baited trap. Since there was no advantage of using treated nylon strips, the cartridge was used for all further experiments.

Exploring the effectiveness of replacing CO_2 with 2-butanone as supplement in MB5-baited Suna traps for the attraction of host-seeking *An. arabiensis*

Experiments were implemented in the absence of a human volunteer with traps set up in competition. There was a strong association between the proportion of mosquitoes recaptured and the test (CO_2, 2-butanone, or nothing). The odds of catching a mosquito in an MB5-baited trap supplemented with 2-butanone was over 30-fold lower than the odds of catching a mosquito if the trap was baited with CO_2 from fermentation (Table4A). The CO_2 supplemented trap recaptured around 36% (95% CI 32-39%) of the released *An. arabiensis* females whilst the 2-butanone supplemented trap and the unbaited trap without any supplement recaptured well below 0.5% of the released mosquitoes (Figure5B).The low catching efficiency of an MB5-baited Suna trap supplemented with 2-butanone was similar in choice tests where the 2-butanone trap was tested in presence of a CO_2 trap and where the 2-butanone trap was tested in presence of a completely unbaited trap (p=0.337; Figure5C).

Notably, the attraction of an odour-baited Suna-trap appears to be largely due to the inclusion of only fermentation-basedCO_2. The chemical blend (MB5) appeared to add very little(CO_2 only vs. reference of MB5 plus CO_2: OR 0.73; 95% CI 0.64-0.84; Table 4)to the attraction of host-seeking *An. arabiensis*.

Testing the effectiveness of MB5-baited Suna traps as pull devices for trapping *An. arabiensis* in close vicinity of a human blood host.

Neither of the MB5-baited traps, either supplemented with CO_2or with 2-butanone, performed well in the presence of a human blood host (Figure6). Whilst in the absence of a human, the MB5-baited Suna trap supplemented with CO_2 recaptured at least half of what was recaptured by a human volunteer (Figure5A), hardly any host-seeking *An. arabiensis* were trapped in the presence of a human blood host (Figure6).

Table 4: **Model outputs for experiments aiming to (A) investigate the performance of 2-butanone and CO_2 in Suna traps; and (B) evaluate the Suna trap in presence of a human blood host.**

Explanatory variables	Odds Ratio (OR)	Confidence Interval (CI)		p-value
		Lower CI	Higher CI	
A. Exploring the association between 2-butanone or CO_2 supplement to Suna traps and the proportion of released *An. arabiensis* trapped				
MB5-cartridge baited Suna trap supplemented **with CO_2**	1			
MB5-cartridge baited Suna trap supplemented **with 2-butanone**	0.03	0.03	0.04	<0.001
Unbaited Suna trap (no MB5, no CO_2, **only suction fan**)	0.01	0.00	0.01	<0.001
Suna trap **baited with CO_2 only** (no MB5)	0.73	0.64	0.84	<0.001
B. Exploring the association between the proportion of released *An. arabiensis* recaptured by human landing volunteers and the presence of a pull device				
HLC in presence of MB5-baited Suna trap and CO_2	1			
HLC in presence of MB5-baited Suna trap and 2-butanone	0.95	0.86	1.06	0.374
HLC in presence of an unbaited Suna trap unbaited fan	1.06	0.98	1.15	0.170

A total of 6901 host-seeking mosquitoes were collected by HLC and traps over all experimental nights, out of which only 1.5% (n=103) were trapped in the Suna traps, whilst the remaining 98.5% were attracted to the human landing volunteers. Consequently, the proportion of host-seeking mosquitoes landing on the volunteer was not affected by the presence of a trap in the system (Figure6A). The proportions landing were equally high in systems where there was only an unbaited trap, in systems where the trap was baited with MB5 and 2-butanone as well as in systems where the trap was baited with MB5 and CO_2 from sugar fermentation (Table 4B).

Experiment 4: Investigating the impact of a complete push-pull set-up

The push system consisting of the 2.5g/m^{2}transfluthrin-impregnatedhessian fabric placed around the eave gaps of the experimental hut was combined with the MB5-baited Suna trap supplemented with CO_2. This was the only pull treatment that was effective in attracting mosquitoes in the absence of a human. The combination was tested since it was considered plausible that the spatial repellent might mask the human odour and hence the trap serving as pull might be more effective than when tested in the presence of a human without the push component.

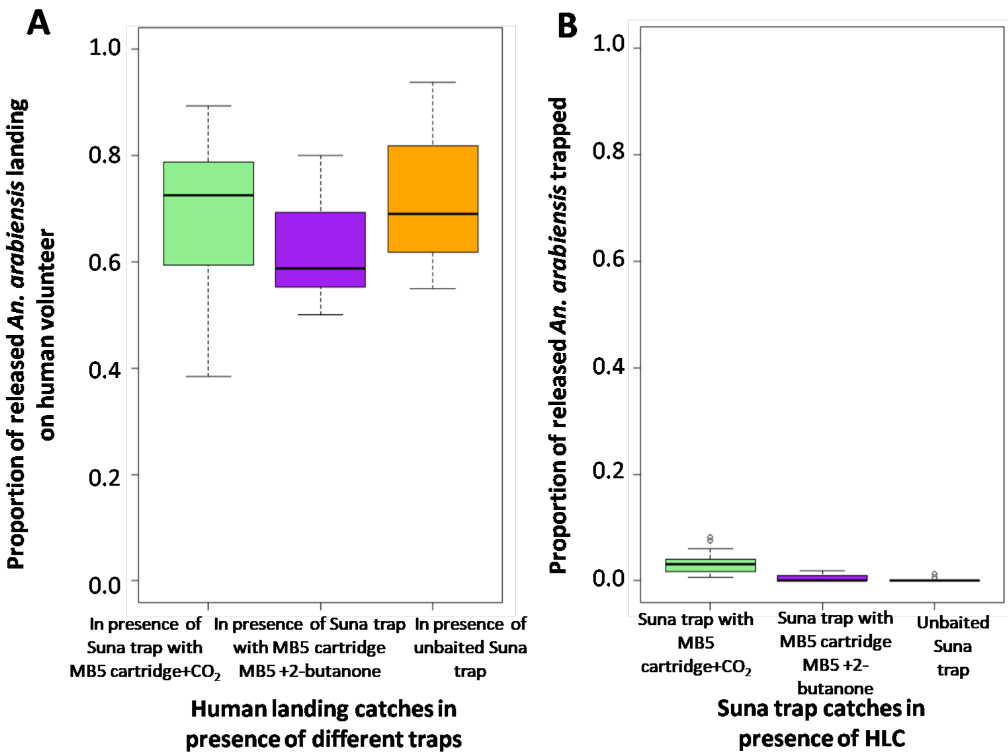

Figure 6.Exploring the attractiveness of MB5-baited Suna traps as pull devices for trapping *An. arabiensis* in close vicinity of a human blood host.(A)Comparing the attraction of mosquitoes to human landing volunteers in the presence of Suna traps either baited with MB5 & CO_2, MB5 & 2-butanone or unbaited with only the fan running. **(B)**Comparing the attraction of mosquitoes to the different traps in the presence of the human blood host. A total of 160 host-seeking mosquitoes were released in the semi-field system per experimental night. HLC was done for four hours (19.00-23.00 h) and the traps ran over night for 12 hours.

Comparing the functional push-pull set up with the set up containing all components but without chemicals (untreated), the odds of receiving a mosquito landing to bite was 3.4 times lower in the presence of the push-pull system (OR 0.29 (95% CI 0.25-0.34), p<0.001; Figure 7). However, this result needs to be interpreted with caution since the reference set up here (all components without chemicals) already presents an intervention which was associated with increased outdoor biting as compared to the control where all components were absent. The presence of untreated and unbaited components was associated with a higher odds of a mosquito landing on a volunteer (OR 2.22 (95% CI 1.96-2.52), p<0.001) compared to the setting where all components were completely absent. It is unclear if this might be due to the fabric strips preventing mosquitoes from entering the hut, hence keeping them closer to the human, or if the observation might be due to other unaccounted conditions given that the two control experiments were implemented at different time points (Figure 7).A more conservative approach in estimating the impact is therefore to compare the odds of a mosquito trying to bite a human volunteer between the push-pull system and the control without any intervention. In this case, the odds was 1.7 times lower in the presence of the repellent-treated and odour-baited push-pull system than in the control (OR 0.59 (95% CI 0.52-0.66), p<0.001). Notably, the estimated reduction in the proportion of *An. arabiensis* landing on the human host in this combined push-pull experiment was much lower than it was in experiment 2 when the push was tested alone (2.5 g/m^{2}transfluthrin fabric compared to untreated fabric OR 0.17 after 1 week post-treatment).

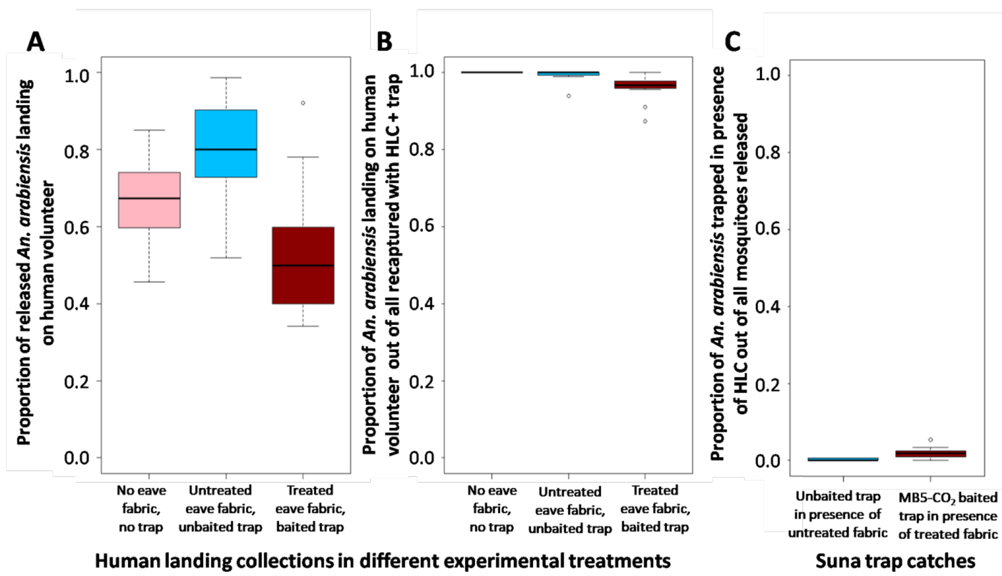

Figure 7.Exploring the impact of a complete push-pull set-up on *An. arabiensis* landing on a human volunteer or being attracted to a trap.(A) Proportion of released mosquitoes landing on the human volunteer outside the hut in the presence of a Suna trap. **(B)** Proportion of mosquitoes collected while landing on the human volunteer out of all mosquitoes recollected total of HLC and trap catches). **(C)**Proportion of mosquitoes trapped in unbaited or baited Suna traps (in presence of HLC) out of all mosquitoes released. A total of 160 host-seeking mosquitoes were released in the semi-field system per experimental night. HLC were done for four hours (19.00-23.00 h) and the traps ran over night for 12 hours.

Temperature and relative humidity variations during experiments

During all experimental set ups, the temperature and relative humidity were logged to determine possible variations between experiments and impact on experimental output. The mean temperature during the experimental hours for the baseline experiment(open eaves, no trap) was 23.6°C (95% CI 23.4-23.7), while it was for the pull-only set ups 24.7°C (95% CI 24.6-24.8). The mean temperature during the final push-pull experiment was with a mean of 22.2°C (95% CI 22.1-22.3) nearly one degree lower that during the push-only experiment with transfluthrin 23°C(95% CI 22.8-23.1). The relative humidity was maintained at >70% during the experimental hours throughout the different experimental set ups.

Detection of volatile chemicals released by the push-pull components

Air movement and temperature variations during chemical quantification

Air samples for chemical analysis were taken in August during the dry season and the average temperatures during the sampling hours were 20.7°C (95% CI 20.4-21.0°C). The air-samplings were done in relatively still air. Air movement was recorded every 10 minutes during the sampling times and most of the recordings (75%) indicated 'no movement'. During the remaining times a low air speed (0.6-1.7 m/s) was measured, consistently from a North East to East North East direction (45°-66°). This meant as indicated in Figure 2, that at the sampling location the air moved from the direction of the hut towards the sampling points.

Detection and estimated concentration of transfluthrin

Transfluthrin was detected at all sampling points. The concentrations decreased greatly with distance from the release point (positions A, B, C and D; Figure 12) with the highest concentration detected nearest to the point of release (position A), and the lowest concentration being detected farthest away from the point of release (position D). Variations in concentrations at different heights were seen across all the sampling positions ($p=0.03$)with a general trend for higher transfluthrin concentrations being found at lower sampling points of≤1m from the ground (Figure8). The averaged concentrations of transfluthrin at position A (nearest to experimental hut) and position D (nearest to Suna trap) were significantly different ($p=0.02$; Figure 12) as were the concentrations at positions B and D ($p=0.002$; Figure8). The highest concentration of transfluthrin detected was 26.3 ng/l (95% CI 21.6-31.0 ng/l) at 1 m from the release point (position A) at 0.5 m from the ground. At 1.5m of the same sampling position, the transfluthrin concentration was 5.7ng/l (95% CI 3.1-8.2ng/l). The lowest concentration was 1.7 ng/l (95% CI 1.2-2.3 ng/l), detected 4 m away from the release point (position D) 1.5m above the ground.

Detection and estimated concentration of MB5 constituents

Two of the five MB5 constituents namely L-(+)-lactic acid and ammonia solution were not detectable under the analytical conditions since the stationary phase of the column used was for the detection of non-polar compounds while the two compounds are polar in nature (Short *et al.*, 1983; Hasegawa *et al.*, 2008). The remaining compounds, namely 3-methyl-1-butanol, butan-1-amine and tetradecanoic acid were detected at low concentrations in some, but not all samples.

Of the 60 samples collected, 3-methyl-1-butanol was quantified in only 15 (25%), with the rest falling below the detection limit. The highest concentration of 0.4 ng/l (95% CI 0.07-0.75 ng/l) was detected closest to the release point at position D, 1 m from the Suna trap. Contrasting to all other chemicals in the push-pull system, 3-methyl-1-butanol was found at the highest average concentration at 1.5m above the ground. At the same position, the average concentration was0.13 ng/l (95% CI 0-0.38 ng/l) at 1.0 m above ground and 0.04 ng/l 95% CI 0-0.13ng/l)at 0.5 m.

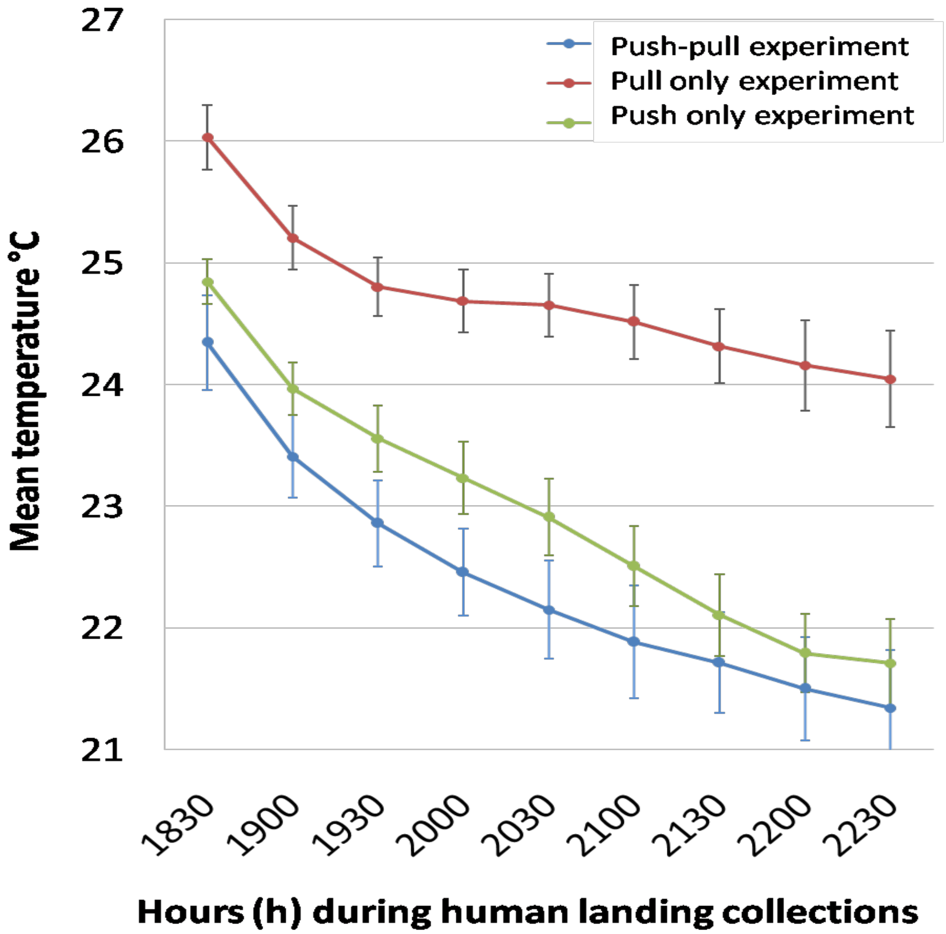

Figure 8. **Hourly mean air temperature in the semi-field systems during the human landing collections (19.00-23.00 h) of different experiments.** The data variability is shown with 95% confidence intervals.

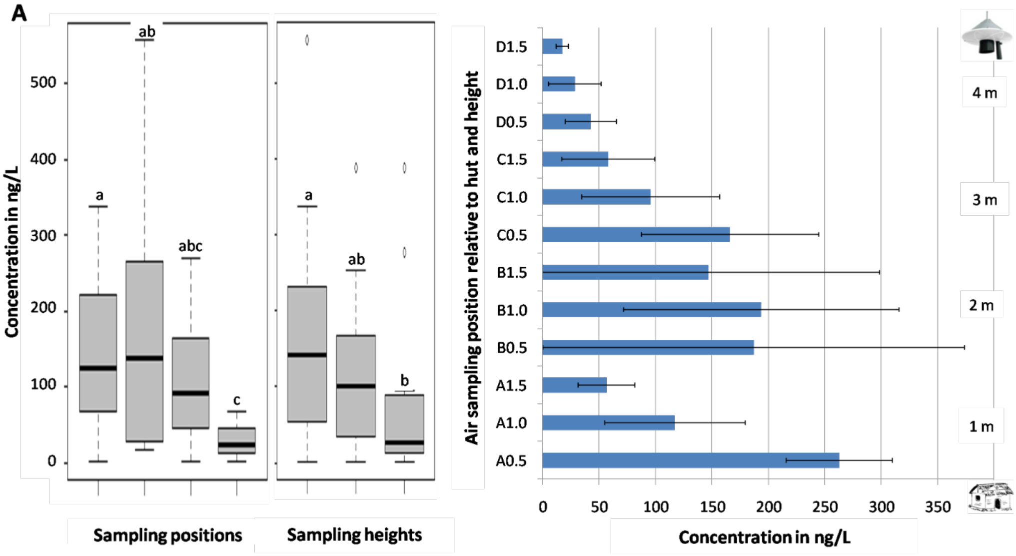

Figure 9. Transfluthrin concentrations estimated from air-sampling at different distances and heights. (A) Median concentrations in nanograms per litre of transfluthrin across the four sampling positions from A (1 m from release point) to position D (4 m from release point) as well as across sampling heights from 0.5m above the ground to 1.5 m. **(B)** Mean transfluthrin concentration (Standard Error bars) in nanograms per litre of air sampled for every sampling point.

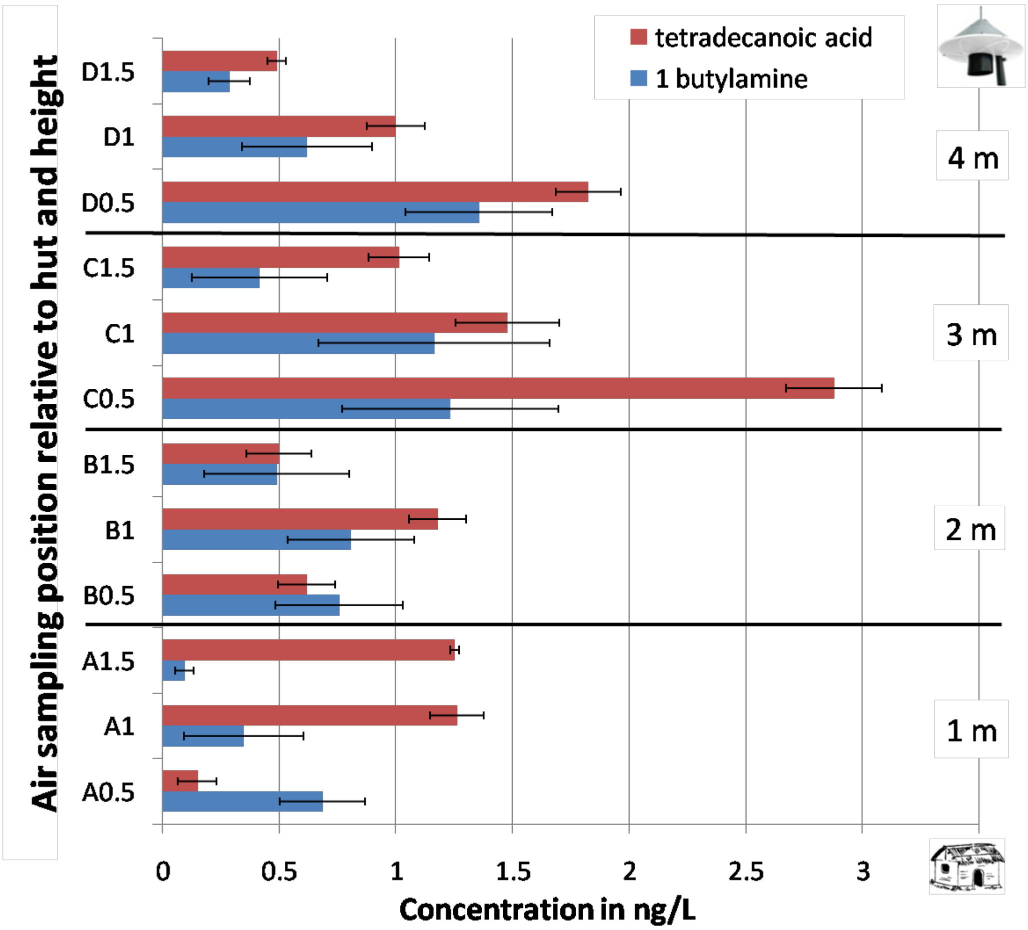

Figure 10. Average concentrations in nanograms per litre (standard error bars) of 1-butylamine and tetradecanoic acid across all air-sampling positions.

Out of the 60 air samples, 1-butylamine was detected in 31 samples (52%), while tetradecanoic acid was detected in 42 samples (70% of samples). There was no strong association with distance and height for these chemicals, though some trends can be observed from Figure9. The chemical 1-butylamine was consistently detected at higher concentrations closer to the ground (≤1 m). This also applied to tetradecanoic acid, at least within 2 m from the release point (positions D and C). Generally, both chemicals were detected at significantly higher concentrations within 2 m of the releasing trap than further away.

Discussion

The results of this study provide essential insight into the behaviour of host-seeking *An. arabiensis* in response to tools aimed at manipulating their odour-orientation and consequently at reducing the number of potentially infectious bites in the peri-domestic area.

Transfluthrin-treated hessian fabric strips loosely fixed around eave gaps prevented, depending on the experimental conditions, between 40-80% of the *An. arabiensis* bites a human volunteer would have received in the absence of the treatment. On the contrary, the microencapsulated Citriodiol® did not show any spatial repellent properties as concluded from the unaffected human landing rates.

For *An. arabiensis* under the test conditions the components tested for pulling vectors in an attract and kill approach did not perform to expectations based on previous work (Takken *et al.*, 1997; Takken *et al.*, 1999; Okumu *et al.*, 2010b; Hiscox *et al.*, 2014; Mweresa *et al.*, 2014; Mweresa *et al.*, 2015). The Suna trap baited with the MB5 odour-blend and supplemented with CO_2 from molasses fermentation attracted only a third of the released host-seeking females when no human host was in the vicinity, confirming similar studies (Hiscox *et al.*, 2014; Mburu *et al.*, 2017), however, when a human volunteer was in the same system, the trap caught less than 1% of all released mosquitoes whilst a human consistently attracted over two thirds of all mosquitoes released. This was the case in the absence ('pull' only) and in the presence of a spatial repellent ('push-pull' set up).In its current configuration the tested 'pull' did not provide a valuable addition to a spatial repellent in a push-pull system for prevention of *An. arabiensis* bites. At closer scrutiny, in the absence of a human volunteer, it was found that the MB5 odour blend did not add much additional attraction to the trap, and it might be sufficient to only provide CO_2 from molasses fermentation as bait. This confirms data shown graphically from field indoor trap collections of *An. arabiensis*(Mburu *et al.*, 2017)in western Kenya though the authors did not discuss this observation. Our experiments also clearly indicated that 2-butanone is not a suitable CO_2 replacement for the collection of *An. arabiensis* confirming previous observations under similar experimental conditions (Mburu *et al.*, 2017).

Contrary to the majority of recent experimental studies with malaria vectors and odour-baited traps, here we included a human being in the system, since ultimately; a push-pull intervention aims to directly protect people from bites and hence would require traps to compete well with the human host odour when placed in close vicinity. However, human beings remain more attractive to host-seeking malaria vectors despite all the efforts employed in identifying host-seeking cues and to produce synthetic lures due to the high complexity in mosquito host-seeking behaviour (Okumu *et al.*, 2010a; Carde, 2015; van Breugel *et al.*, 2015; Hawkes *et al.*, 2017). Our results support the findings of Okumu et al. who observed that the chemical odour blend attracted host-seeking *An. arabiensis* in representative numbers in the absence of a human, but when presented with the two odour sources side by side within the same hut in field settings, the mosquitoes retained their preferences for humans(Okumu *et al.*, 2010b). The authors suggested that preferences are dependent upon whether the stimuli are in direct short-range competition or whether they are far apart with completely separated odour plumes, which might be the best strategy to exploit for mass-trapping interventions (Kline, 2007; Hiscox *et al.*, 2016; Homan *et al.*, 2016).

Due to the increasing insecticide resistance levels in malaria vectors, this study aimed to explore Citriodiol® with its active ingredient PMD, as a spatial repellent since it belongs to a different class of chemicals than those currently used in public health. It is a well-known topical repellent (Barnard *et al.*, 2002; Carroll *et al.*, 2006; Colucci *et al.*, 2018; Lee *et al.*, 2018; Carroll *et al.*, 2019; Afify *et al.*, 2020)and has been suggested as having spatial repellent properties in a previous study(Menger *et al.*, 2014). However, the previous evaluation was done using electricity-powered active emanators to dispense the chemical. Furthermore, the product was not microencapsulated but applied as Citriodiol® oil at high concentrations on a nylon strip and several emanators used in a semi-field system less than a quarter of the size of those used here(Menger *et al.*, 2014). Such effort is neither operationally feasible, nor cost-effective. Importantly, the previous study did not include a human blood host in the test system but used an MB5-baited trap as a substitute(Menger *et al.*, 2014). We opted for microencapsulation to secure the Citriodiol® into the fabric with the aim to allow passive slow-release of the repellent for possible long-term usage when fixed on eave gaps(Onder *et al.*, 2008; Misni *et al.*, 2017).However, neither of the two test concentrations resulted in any protection against mosquito bites, not even when the material was fixed very closely to the human volunteer on the chair (data not shown). Optimal formulation and presentation of a repellent, whether spatial or topical, is key for effectiveness (Carroll *et al.*, 2019) and further work might be warranted. For now, it remains unclear, if the concentrations of chemicals released from the fabric were just too low, or whether PMD does in fact have no spatial repellent properties.

Transfluthrin is a pyrethroid insecticide which is not only known for its killing effect but also its moderate volatility which makes it an effective spatial repellent (Ogoma *et al.*, 2012b; Wagman *et al.*, 2015a; Nentwig *et al.*, 2016; Mmbando *et al.*, 2018). Transfluthrin has been incorporated into commercial products for mosquito control with encouraging outcomes (Ramesh *et al.*, 2001; Pates *et al.*, 2002; Hill *et al.*, 2014; Jeyalakshmi *et al.*, 2014; Ogoma *et al.*, 2014a; Ogoma *et al.*, 2014b; Maia *et al.*, 2016). Applied on hessian material for passive emanation, it has been proposed to protect from 70-90% of bites from Afrotropical malaria vectors in a range of experimental laboratory and field studies implemented in coastal and inland Tanzania(Ogoma *et al.*, 2012b; Ogoma *et al.*, 2017; Mmbando *et al.*, 2018; Mmbando *et al.*, 2019; Mwanga *et al.*, 2019). Our results confirm the potential of transfluthrin for use as a spatial repellent vector control tool. However, the protective efficacy under most of our test conditions, was much more moderate than in the Tanzanian studies. One reason for this might be the differences in average temperatures during experiments in the different regions(Russell *et al.*, 2010; Ogoma *et al.*, 2012a; Andres *et al.*, 2015; Ogoma *et al.*, 2017; Bibbs *et al.*, 2018). During the implementation of our final push-pull experiment, only around 40% of the bites that would have been received without protection were averted. This was only half the protection we found for the 2.5 g/m^2transfluthrin treatment during the push-only experiment. Given that the pull-only experiments did not provide any evidence that the presence of the odour-baited trap might increase the proportion of mosquitoes attempting to bite the human volunteer, other factors are likely responsible for the lower protection from the spatial repellent at the time. Notably, during the push-pull experiment, evening temperatures were an average of 22 °C, around 1 °C lower than during the push-only experiment. Increases in temperature increase the effective vapour pressure of a chemical and therefore the volatilization rate(Jensen *et al.*, 2007). Cooler temperature conditions lead to lower transfluthrin evaporation rates and it has been previously suggested that the protective efficacy of passively emanated transfluthrin from hessian fabric reduces when temperatures are below 23°C (Ogoma *et al.*, 2017).Conversely, increasing temperatures were associated with increasing airborne transfluthrin concentrations in closed test systems (Pettebone, 2014)and an increase of mosquito mortality with an increase of airborne transfluthrin(Martin *et al.*, 2020).

Our samples for quantification of transfluthrin in the air within 5 m from the release point were taken during the cold season with temperatures during sampling of around 21 °C. Nevertheless, the chemical was consistently detected with concentrations decreasing by an order of magnitude from over 20 ng/l to 1.7 ng/l over five metres from the release point. These concentrations are significantly higher than those reported by Ogoma et al. (Ogoma *et al.*, 2017) who reported 0.13 ng/l from samples collected indoors from a non-ventilated 30 m^3 room, however, the treatment load of the hessian test material was also three times lower (0.8 g/m^2) than in our study. Our estimated concentrations are, nonetheless, well below the maximum acceptable exposure concentration for long-term inhalation exposure of human beings of 500 ng/l, as defined by the regulatory authorities of the European Union (Weinholt, 2016) Our findings relate well with a more recent study using a similar approach to ours on malaria vectors in Vietnam, where airborne transfluthrin concentrations were estimated at 1.32 ng/l at 4 metres from the release point(Martin *et al.*, 2020) and were below the detection limit further away. This study also showed higher knock-down and mortality rates for caged mosquitoes at ground level than above 1 metre from the ground (Martin *et al.*, 2020) supporting our observation of highest concentrations at 1.0 m and below. This might limit the 'protective bubble' especially in the outdoor environment around the house.

The inconsistent detection of the constituents of the putative attractant MB5 in the air, might suggest that the odours were not sufficiently released and dispersed, specifically 3-methyl-1-butanol was rarely picked up by the adsorbent filters. Tetradecanoic acid and 1-butylamine were detected more frequently, though not consistently and at very low concentrations in close vicinity to the trap. Whether the chemical release rates and hence performance of the MB5-baited Suna trap might also be affected by night-time temperatures during trapping needs further investigations. Mechanisms to increase the released concentrations and improve the dispersion might be explored in future by modifying the Suna traps(Verhulst *et al.*, 2015)or surveying alternative traps and baits (Menger *et al.*, 2015; Batista *et al.*, 2017; Hawkes *et al.*, 2017; Batista *et al.*, 2018; van de Straat *et al.*, 2019).A recent study for example suggested the combination of transfluthrin treated fabric on eave gaps with BG Malaria traps (Biogents, Germany) and suggested a larger distance of the pull trap from the human host, nevertheless the authors also found only a very marginal addition of protection from the traps in preventing *An. arabiensis* bites(Mmbando *et al.*, 2019). Strategies combining mass-trapping of mosquitoes with spatial repellents at independent locations rather than combining on household levels should be explored in future.

Finding a pull component that is efficient enough to attract mosquitoes even when a human is close by, yet easy to set and maintain, remains desirable to remove adult vectors from the transmission setting. The idea to develop a push-pull system with the MB5-baited Suna trap was inspired by the successful mass-trapping field trial in western Kenya with the pull component only, which was associated with significant reductions in vector densities (Hiscox *et al.*, 2016; Homan *et al.*, 2016). However, when analysed by vector species, only *An. funestus* densities were reduced in the study site whilst *An. arabiensis,* which accounted for around a quarter of the vector population, were not affected, resulting in only a moderate reduction in malaria parasite prevalence in the study area (Hiscox *et al.*, 2016; Homan *et al.*, 2016). This suggests a species-specific attraction to the MB5-baited trap. Similar observations were made by Mburu et al.(Mburu *et al.*, 2017)when investigating the use of 2-butanone as a CO_2 replacement in a rice irrigation area in western Kenya, where *An. funestus* is the predominant vector species.

Conclusions

This detailed step-by-step evaluation of the selected putative repellent ‘push’ and attractive ‘pull’ components has led to a better understanding of their prospect to affect the host-seeking behaviour of the malaria vector *An. arabiensis* in the peri-domestic space and helps to gauge the impact such tools would have when used in the field for vector monitoring or control.

Chapter 2

This study has highlighted the need for testing odour-based interventions in the presence of a human host to gain accurate estimates. A trap cannot substitute a human being when changes in attraction and human landing rates are the outcome measure. Additionally, the importance of working with different vector systems has been elucidated. There is urgent need to further study potential differences in odour-orientation between the two major vector species complexes*: An. gambiae* s.l. and *An. funestus* group. Overall, it will be desirable to develop odour-baited traps that target all major vectors at the same time for use in varied eco-epidemiological systems.

This study further confirmed that, at least under standardised experimental conditions, that passive emanation of transfluthrin from treated hessian fabric strips around eave gaps can provide protection from mosquito bites in the peri-domestic space. Comparisons across published work have, however, also highlighted that the expected impact might be quite variable from location to location, depending on climate conditions and vector species. Data generated under standardized field conditions in a single location needs to be interpreted in the local context and should be replicated under different conditions to ensure recommendations can be generalised or can be tailored to local contexts. Mathematical modelling can support decision making by integrating data from different settings in prediction models to understand the geographical range where such tool might be useful and the impact to be expected under a varying environmental and epidemiological conditions. Field evaluations are required to investigate how results from semi-field experiments correlate to findings from field trials. For example, air movement was minimal in the semi-field systems and the repellent transfluthrin was detected within 5 m from the experimental hut. However, it must be assumed that this is quite different from natural conditions, especially during rainy seasons when vector densities and malaria transmission peak. Rainstorms characterising the tropical evenings might well interfere with the odour plumes and protective bubble around the house. One might therefore plausibly assume that the protective efficacy in the peri-domestic space in western Kenya field sites would be lower than the largely moderate effects observed in the current experiments.

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Declarations

Ethics approval and consent to participate

This study was approved by the Kenya Medical Research Institutes Scientific and Ethics Review committee (KEMRI-SERU), protocol number NON-KEMRI 546.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AH, AS, SM and WT conceived the initial idea for this research and secured the funding. AH, AS, SM, UF and MMN jointly developed the experimental designs for the experiments. MMN, UF and AH developed experimental standard operating procedures. MMN implemented the experiments. MMN and UF analysed and interpreted the data and drafted the manuscript with support from AH, WT and JvL. All authors contributed to the final draft. All authors read and approved the final manuscript.

Chapter 3

Sight versus smell: Dynamics of malaria mosquito attraction response in the presence of spatial repellents

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Chapter 3

Abstract

Mosquito behaviour is influenced by perception of and response to cues that provide information on host location and quality. Olfaction plays a central role in this process, with volatile attractants and repellents affecting mosquito behaviour. Strategies to manipulate the odour orientation of mosquitoes are currently under development to support vector control efforts for diseases vectored by mosquitoes such as malaria. Spatial repellents appear to be a promising new tool for vector control, but in order to prevent diversion of bites to unprotected persons, it would be beneficial to combine the spatial repellent with an attractant-based trapping method. Here we explore if and how the spatial repellent transfluthrin affects the trapping efficacy of *Anopheles arabiensis* using attractant-baited Suna and CDC UV light traps, both supplemented with CO_2, under semi-field conditions and compare with that of *An. gambiae s.s*. Dual choice set ups were conducted in screen houses containing experimental huts with eave fabrics treated with spatial repellent or untreated. In the absence of a repellent, dual choice set-ups showed a relative 1:1 distribution of all trapped mosquitoes between the two trap types, although the proportion of trapped mosquitoes was less than 1/3 of all released. Trapping efficiency of an odour-baited Suna trap for *An. arabiensis* was reduced 2.3 times in the presence of a transfluthrin-treated eave fabric compared to when the odour-baited Suna trap was used in the presence of untreated eave fabric. CDC UV light traps trapping efficiency calculated as recaptured proportion of released mosquitoes remained relatively constant both in the presence and in the absence of the spatial repellent. Trapping efficiency for *An. gambiae S.S*. was not significantly different to *An. arabiensis* though fewer *An. gambiae* s.s. were trapped in odour-baited Suna trap ($p=0.767$) and more caught in the CDC UV light trap ($p=0.178$) in the presence of the repellent. The results showed that in very close proximity to a repellent, a CDC UV light trap would be preferred instead of an odour-baited Suna trap as a suitable inclusion in a combination set up for the control of malaria vectors though a more suitable alternative pull component should be considered.

Key words:

Anopheles arabiensis, Spatial repellent, CDC UV light trap, Olfaction, Eave-fabric, Dual-choice

Chapter 3

Introduction

Understanding host-seeking responses of mosquitoes to chemical and physical cues is key to the development and improvement of tools aimed at vector surveillance and the management of diseases mosquitoes transmit (Mbogo *et al.*, 1993; Magbity *et al.*, 2002; Kline, 2007). Odour-baited traps targeting host seeking or egg-laying vectors were developed by studying mosquito biology and identifying the cues that mosquitoes utilize for locating a blood host or oviposition site (Takken *et al.*, 1997a; Spitzen *et al.*, 2008; Turner *et al.*, 2011; Dugassa *et al.*, 2013; Revay *et al.*, 2013; Wong *et al.*, 2013; Hiscox *et al.*, 2014; Wagman *et al.*, 2014). The long-term success of control tools reliant on insecticides has recently been questioned due to increased insecticide resistance among malaria vectors to all major groups of insecticides (Agossa *et al.*, 2015; Liu, 2015; Sande *et al.*, 2015; Hancock *et al.*, 2020). This has contributed to the stalling in decline of malaria cases and, in some areas, resurgence of malaria, emphasising the need for development of supplementary tools for enhanced control (WHO, 2019).

Recent developments in vector control have explored the use of insecticides used at sub-lethal concentrations as spatial repellents to divert host-seeking mosquitoes away from the potential human blood host and hence aim for a reduction in infective bites (Hill *et al.*, 2007; Achee *et al.*, 2012; Revay *et al.*, 2013; Maia *et al.*, 2016; LeClair *et al.*, 2017). Use of spatial repellents, such as transfluthrin for conferring outdoor protection, holds a lot of promise though there is the potential risk of diverting mosquitoes to unprotected populations (Achee *et al.*, 2009; Achee *et al.*, 2012; Maia *et al.*, 2016; Ogoma *et al.*, 2017; Maia *et al.*, 2018). To counter this, the use of spatial repellents in combination with traps that attract and kill the vector has been proposed (Takken, 2010; Wagman *et al.*, 2015).

Host-seeking behavioural responses in *Anopheles* mosquitoes have been largely attributed to the perception of carbon dioxide (CO_2) gradients and a combination of human odours, moisture and warmth that the mosquitoes use to orient themselves to a blood host (Healy *et al.*, 1995; Takken *et al.*, 1997a; Takken *et al.*, 1997b; Gibson *et al.*, 1999; Okumu *et al.*, 2010c; Turner *et al.*, 2011; Ray, 2015). Identification of specific host-odours associated with host-seeking has led to the development and utilization of synthetic lures used in traps (Okumu *et al.*, 2010b; Mukabana *et al.*, 2012a; Hiscox *et al.*, 2014; Hiscox *et al.*, 2016; Homan *et al.*, 2016). Using an odour-baited trap in combination with a spatial repellent for the control of outdoor-biting mosquitoes could potentially trap repelled mosquitoes and remove them from the environment instead of redirecting them to unprotected populations (Menger *et al.*, 2014; Menger *et al.*, 2016). However, studies with *Aedes albopictus* have shown that exposure to spatial repellents (geraniol, citral, eugenol and anisaldehyde) leads to a reduction in host-seeking behaviour

(Hao*et al.*, 2008). Others found that repellent insecticides induce an irritant reaction leading to reduced feeding in *Anopheles* mosquitoes due to avoidance behaviour (Chareonviriyaphap *et al.*, 2004; Hodson *et al.*, 2016). Overall, there is evidence that repellents affect the odorant receptors of dipterans by affecting the ability of the insect to perceive attractive chemicals (Bohbot *et al.*, 2010; Hodson *et al.*, 2016; Afify *et al.*, 2020) which would be counter-productive in a combined push-pull intervention, particularly where the pull device is in proximity to the push device. Indeed, DEET has been shown to reduce the perception of human odours by *An. gambiae* olfactory receptor neurons (Ditzen *et al.*, 2008). The attractiveness of a lure in an odour-baited trap within the action range of a spatial repellent may be masked similarly to the odours from the natural blood host which should be protected from bites. Such an effect would render the trap less effective than intended (Achee *et al.*, 2012). Consequently, it is necessary to investigate how receptive mosquitoes exposed to repellents are to simultaneous exposure to potential chemical lures and alternative non-chemical cues.

Mosquito perception of light has been shown to aid in most of their biological activities including visual perception of blood hosts (Allan *et al.*, 1987; Lehane, 2005 ; McMeniman *et al.*, 2014). While mosquitoes are nocturnal in nature and usually feed when dark (Wamae *et al.*, 2015), light plays a major role in modulating their behaviour (Rowland, 1989). Exposure to short bursts of bright white light for example, prior to the start of the dark phase of the daily cycle, greatly reduced their host-seeking ability (Sheppard *et al.*, 2017).

Mosquitoes generally respond to light intensity levels by initiating flight activity followed by orientation based on visual perception towards an intended target (Allan *et al.*, 1987). To some extent, moonlight affects mosquito behaviour by triggering increase in flight in *An. taeniorhynchus* (Bidlingmayer, 1964). Host-seeking *Aedes* spp. and *Mansonia perturbans* female mosquitoes are drawn more to low intensity light such as blue, red and black than to high intensity ones such as yellow and white (Browne *et al.*, 1981). Like most vertebrates and invertebrates, mosquitoes have UV-sensitive rhodopsins, with *An. gambiae* photoreceptors specifically those that possess Agop8 rhodopsins, being shown to be particularly important in the detection of UV light (Hu *et al.*, 2014). As all mosquitoes require sugar meals for energy, it has been shown that some flowers have UV-reflecting guides (Silberglied, 1979) which mosquitoes, many of whose UV receptors are sensitive in the 340-360nm range, follow to obtain nectar (Stark *et al.*, 1982). The role of light in mosquitoes which are in the host-seeking phase of their gonotrophic cycle works in tandem with olfactory perception. This was seen in a study that showed how *Ae. aegypti* detection of CO_2 activated a significant attraction to visual features which prompted the mosquito to move closer to the potential host in order to detect other shorter-range cues such as host volatiles and heat (van Breugel *et al.*, 2015). Light traps utilize this perception and attraction to light of different wavelengths with the combination of odour stimuli and air currents for monitoring and surveillance of mosquito populations (Joshi *et al.*, 1975; Fornadel *et al.*, 2010; González *et al.*, 2016; Ponlawat *et al.*, 2017b). The performance of light traps in attracting and killing mosquitoes has been shown, in many cases, to be species-specific (Wong *et al.*, 2013; Wagman *et al.*, 2014; Mburu *et al.*, 2019). The majority of commercial light traps employ the use of 4-6 W incandescent bulbs that produce a broad visual spectrum of light that goes beyond the visible light range (Evans *et al.*, 2009). Changes in specification of the light wavelength to more specific wavelengths have improved trapping efficiency for different mosquito species. UV light has been shown to be superior to incandescent light in trapping mosquitoes (Moore *et al.*, 2001; Li *et al.*, 2015b).

In the present study the trapping efficiency of CDC UV light traps was compared with the efficiency of MB5-baited Suna traps supplemented with CO_2 in the presence and absence of a spatial repellent under semi-field conditions. Mosquitoes released in the semi-field system were either momentarily or continuously exposed to transfluthrin as a spatial repellent. The sibling species *An. gambiae s.s.* and *An. arabiensis* were compared to investigate any species-specific responses.

Materials and methods

Experimental site

Small semi-field systems located at the International Centre of Insect Physiology and Ecology – Thomas Odhiambo Campus, Mbita, western Kenya (-0.430356, 34.206416) were used for all the experiments. The systems measured 11.4 m by 7.1 m and were screened with fibreglass netting. The roofs were made of translucent polycarbonate and the soil floors had a top layer of 50 cm of sand (Okal *et al.*, 2015). The floors were watered regularly to maintain a relative humidity between 60% and 70%, an optimal environmental condition for behavioural assays on host-seeking mosquitoes (Benedict, 1997). In each semi-field system, a traditional East African mud hut 3.1 m by 3 m and with a height of 2.2 m, with corrugated iron sheets on the roof, was located at the centre. The hut had one window and one door which were both screened and remained closed throughout the experiments, while the 10 cm wide eave gaps between the roof and wall were open all round (Figure 1). A 5 cm wide hessian fabric (burlap, which was procured from local markets) impregnated with transfluthrin was wrapped around the eave gaps of the hut leaving approximately 2.5 cm gap above and below the fabric.

Mosquitoes

Two hundred female insectary-reared *An. arabiensis* Mwea strain were used for each experimental replicate. In two experiments, comparison of behavioural responses was done using *An. gambiae s.s.* Mbita strain, also reared in the same conditions as *An. arabiensis*. Nulliparous females aged 3-6 d post-

Figure 1. Semi-field system (11 x 7 m) at *icipe*-TOC containing a traditional hut (3 x 3.1 m) made of mud walls and iron sheets for the roof.

emergence were selected from rearing cages. Mosquitoes were reared under ambient environmental conditions with the adults being fed on 6% glucose solution through wicks made from absorbent tissue paper from the time of emergence (Benedict, 1997).

The mosquitoes were selected from the cages into paper cups using hand-held mouth aspirators, by choosing the females that were activated by the presence of a hand close to the side of the cage. They were then starved (no access to water or glucose solution) for 5 h prior to experiments in a holding room that had similar conditions as the insectary.

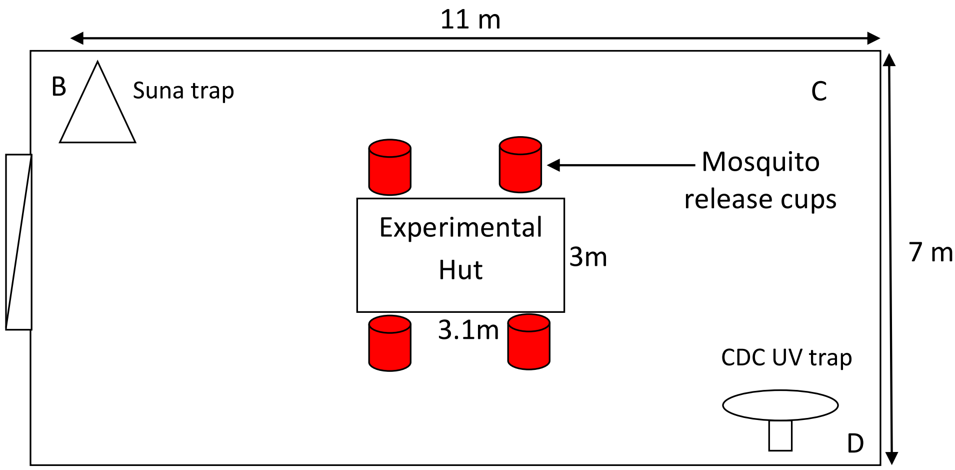

Figure 2. Schematic presentation of experimental design. Traps were placed diagonally opposite each other and were rotated through all corners (A-D) of the semi-field system to remove side bias over the 16 experimental nights. Mosquitoes were released from two sides of an experimental hut from release cups holding 50 mosquitoes each.

Odour-baited Suna trap

The combination of CO_2 and a synthetic odour-blend referred to as the Mbita blend 5 or MB5 (Mukabana *et al.*, 2012b; Mweresa *et al.*, 2014) was used in Suna traps (Biogents, Germany). Their development, structure and *modus operandi* are described elsewhere (Hiscox *et al.*, 2014). The MB5 comprised of five constituent chemicals namely; ammonia (2.5% in water), lactic acid (85% in water), tetradecanoic acid (0.00025 g/l in ethanol), 3-methyl-1-butanol (0.000001% in water), and butan-1-amine (0.001% in paraffin oil) (Mukabana *et al.*, 2012b; Menger *et al.*, 2014; Mweresa *et al.*, 2014). All chemical compounds were purchased from Sigma Aldrich Chemicals GmbH (Germany). Each component of MB5 was dispensed from an individual strip of nylon (measuring 26.5 cm × 1.0 cm). The nylon strips (15 denier microfibers, 90% polyamide, 10% spandex) were purchased from Bata Shoe Company Ltd, Kenya. Baits were prepared and used for a maximum of one experimental run (16 experimental nights) before fresh ones would be prepared. Every evening, the baits would be removed from an -20 °C freezer and hung in the traps for the entire night before careful removal in the morning and placing in a clean aluminium foil and then folded into the foil (without bending the strips) and put back into the freezer. Carbon dioxide was produced by mixing 250 g of molasses (Mumias Sugar Company Ltd, Kenya), 17.5 g dry yeast (Angel® Yeast Company Ltd, China) and 2 l of water (Saitoh *et al.*, 2004; Oli *et al.*, 2005; Mweresa *et al.*, 2014) with a fresh mixture used for each experimental night.

CDC-UV light trap

CDC-UV light traps from Bioquip Products (California, USA) were used for all the experiments. A blue-black light tube and transistorized inverter ballast provided radiation in the near UV range (4 W, ca. 320-420 nm) and was purchased with the trap used in the experiments (Bioquip Inc). The CDC-UV light trap was supplemented with CO_2 (produced from molasses) in the same way as the odour-baited Suna trap to elicit long-range activation of mosquitoes. For both traps, the CO_2 was released from a tube connected near the fan of the trap below the trap cover.

Spatial repellent

Emulsified concentrate (EC) of transfluthrin from Bayer Global (Leverkusen, Germany) at a concentration of 0.2 g/ml was used as spatial repellent. Hessian fabric measuring 21 m by 5 cm (Ogoma *et al.*, 2012) was impregnated by soaking it in transfluthrin EC solution to achieve a final load of 2.5 g/m^2. Preparation of the EC solution involved measurement of water required to sufficiently wet one roll of eave fabric (approximately 1300 ml of water per fabric) and adding 26.25 ml to attain 2.5 g/m^2 as the area of the fabric was 2.1 m^2(Ogoma *et al.*, 2012; Ogoma *et al.*, 2017). This was carried out separately for each strip. Fabric was dried in the shade prior to use. The treated fabric was used continuously for up to 16 nights with the fabric being up 24 h daily (complete single experiment run). In experiments without exposure to transfluthrin (control experiments) or in which mosquitoes were exposed to the spatial repellent briefly before being taken to the semi-field system, untreated hessian fabric of similar dimensions (21 m by 5 cm) were prepared. Treated and untreated hessian wraps were hung in the same way around the eave gaps of the huts.

Experimental set up

All experiments were dual choice tests, where host-seeking *An. arabiensis*(in all experiments) and *An. gambiae s.s.* (in two experiments only) were released in the presence of two traps, either an odour-baited

Suna trap and a CDC-UV light trap or two specimens of the same trap types. The traps were both supplemented with CO_2 at any time. Each trap was mounted on a tripod stand with the mosquito entry point of the trap at 30 cm from the ground (Hiscox *et al.*, 2014). They were placed diagonally opposite to each other. Their positioning was changed nightly following a clockwise rotation around the semi-field system for 16 nights to remove location or side bias (Figure 2). Mosquitoes were released at two sides of the experimental hut from a total of 4 release cups with 50 mosquitoes in each cup (2 cups per side). In the experiments with both mosquito species, 25 specimens of each species were released from a cup with the mosquitoes colour-coded with two different fluorescent dyes; green for *An. gambiae s.s.* and red for *An. arabiensis*. The dusting was done by placing approximately 1 g of fluorescent dye (Fingerprint powder, BVDA International B.V., the Netherlands) in a 10 ml syringe (without a needle) then blasting this into a release cup containing the mosquitoes (Verhulst *et al.*, 2013). Traps were switched on at 18:30 h and mosquitoes obtained from either the holding room or wind tunnel as described below were released at 19:00 h. The traps were removed after 12 collection hours and all mosquitoes in the catch bags from either trap were counted.

Preliminary experiments

Dual choice tests aimed at identifying a preference for one trap type over the other. If both traps are equally attractive to host-seeking mosquitoes (or equally efficient in collecting them), as might be assumed by providing two identical traps, a 1:1 distribution of captured mosquitoes would be expected over the course of the 16 experimental nights. If one trap is more efficient than the other, we expect a significant deviation from the reference 1:1 distribution. In order to understand the dynamics in the semi-field system in terms of trapping efficacy in dual choice tests without any treatments in place, a set of preliminary experiments were implemented (Table 1, experiment 1-3; preliminary experiments). The first set of experiments (experiments 1 and 2) provided two equal traps (2 Suna traps and 2 CDC UV light traps respectively), whilst experiment 3 provided the two different trap types. The second set of experiments was implemented with either untreated or transfluthrin treated hessian fabric wrapped around the eave gaps of the experimental hut in the semi-field system (Table 1, experiments 4-6; effect of repellent on trapping).

Pre-exposure experiment

Mosquitoes were pre-exposed to a repellent immediately prior to release in the semi-field system to determine whether any changes in behaviour may result from a short exposure lasting 5 min. Specimens that had been starved for 5 h were exposed to transfluthrin by placing them in single-use release cups in a wind tunnel for 5 min. in the presence of a 10 cm x 30 cm hessian fabric impregnated with 0.075 g of transfluthrin (load on fabric = 2.5 g/m^2) which was positioned 30 cm from the release cups. The wind tunnel was switched off during the exposure; hence there was no directional airflow. Preliminary experiments confirmed that there was no mortality induced by this exposure for a 12 h post-exposure observation period. After exposure, the treated fabric was removed, wrapped in aluminium foil and stored in a cold room at 2°C for reuse for up to 16 experimental days.

Table 1: Overview of all two-choice semi-field experiments (no. 1-6) to compare the efficacy of trapping released insectary-reared *Anopheles* mosquitoes with odour-baited Suna and CDC UV light traps in the absence and presence of the spatial repellent transfluthrin. All traps were supplemented with CO_2.

Experiments	**Trap 1**	**Trap 2**	**Test species**
Preliminary experiments in absence of fabric around eave gaps			
(1) Reference 1 – 2 equal trap choices (odour vs. odour)	odour-baited	odour-baited	AA
(2) Reference 2 – 2 equal trap choices (UV light vs. UV light)	UV light	UV light	AA
(3) Odour versus light - 2 trap choice	odour-baited	UV light	AA
Experiments in presence of fabric around eave gaps investigating the effect of transfluthrin on trapping			
(4) Odour versus light - 2 trap choice, untreated fabric	odour-baited	UV light	AA, AG
(5) Transfluthrin pre-exposed - 2 trap choice, untreated fabric	odour-baited	UV light	AA
(6) Odour versus light - 2 trap choice, transfluthrin treated fabric	odour-baited	UV light	AA, AG

AA: *Anopheles arabiensis*. AG: *Anopheles gambiae*

The wind tunnel was then wiped down with 70% ethanol and 2-propanol (undiluted laboratory grade) and allowed to dry to prevent accumulation of transfluthrin. Immediately following exposure, mosquitoes were taken for release in the semi-field system which featured an untreated fabric on the eave gaps of the experimental huts (Table 1, experiment 5; pre-exposure). In comparison, unexposed mosquitoes were starved as described before and exposed in a second screen house in parallel to the first (Table 1, experiment 4; unexposed).

Constant exposure experiment. To investigate host-seeking events in the presence of a repellent, transfluthrin-treated hessian fabric was positioned on the eave gap of the experimental hut inside the semi-field system and secured using aluminium wires, ensuring 2.5 cm gaps above and below the fabric. The second semi-field system served as control with an untreated eave fabric positioned in the same way. Test and control were alternated after every 4 nights with a 2 night break in between to ensure that there was no residual effect of the previous treatment. The experiment was replicated over 4 blocks of 4 nights (16 experimental nights, Table 1; experiment 6; exposed).

Data analysis

Data analyses were done using R version 3.5.1 (R Core Team, 2018). All mosquito catches were analysed as proportions, either relative to all mosquitoes released in the semi-field system or relative to all mosquitoes recaptured with the two traps. The total number of mosquitoes caught in both traps was defined as the 'responding' mosquitoes whilst those released but not recollected in either trap were classified as having 'not responded' either because they were not in a host-seeking stage, were killed by predators (e.g. spiders, geckos) before responding or were not attracted/trapped by any of the traps. The dual-choice assays were analysed using generalized linear models with a quasibinomial distribution fitted to account for over-dispersion. The proportions of mosquitoes collected in trap 1 or trap 2 were compared across experiments (dual choice tests implemented in different semi-field systems) in the absence of any treatments for the preliminary experiments and across experiments in the absence or presence of transfluthrin exposure in the experimental set up (Table 1). It was hypothesized that host-seeking females presented with an identical choice would be trapped in approximately equal proportions in both traps. The statistical analysis aimed to reveal if the test treatment of interest (trap) received an increased or decreased proportion of the total number recaptured in an experiment compared to the proportion recaptured in the reference group. Therefore, the experiment ID (Table 1; 1-6) was included in the model as fixed factor to analyse the impact of the experimental conditions on the outcome. The mean proportion of mosquitoes trapped in trap 2 under different experimental conditions and their 95% CIs were estimated based on the model by transforming the log odds (logit) of the outcome to the odds scale and from the odds scale to the probability scale. The data are graphically presented as box and whisker plots showing proportional distribution of trapped mosquitoes in reference traps with the middle line representing the median proportion with the whiskers indicating the maximum and minimum proportional catches.

Chapter 3

Ethical considerations

This study was approved by the Kenya Medical Research Institutes Scientific and Ethics Review committee (KEMRI-SERU), protocol number NON-KEMRI 546.

Results

Preliminary choice experiments (Experiments 1-3, No eave wrap)

Dual choice experiments testing two identical traps were implemented to confirm the performance of the bioassay system; one with two MB5-baited Suna traps and another with two CDC UV light traps in one screen house; all traps were supplemented with CO_2. The third experiment provided a choice between the two different trap types. The proportion of mosquitoes trapped in trap 1 out of the total trapped was not associated with the experiment (Table 2, Figure 3A). Tests in which two identical traps were presented had, as expected, an approximate ratio of 1:1 of mosquitoes trapped by the two traps. A similar 1:1 ratio was observed when two different traps were provided as choice.

The proportional outcome was less variable around the median when the equal choices were odour-baited Suna traps (green box and whisker plot in Figure 3A), as opposed to a much higher variability in the nightly response when two UV light traps, or one UV light trap and one odour-baited Suna trap were used (blue and yellow box and whisker plot in Figure 3A).

Whilst the ratios indicate how the two traps compared, they did not provide insight as to how efficient any of them was in trapping a given mosquito population under semi-field conditions. It was hence important to additionally explore the recapture rate out of all mosquitoes released (as opposed to all trapped) in the relatively small semi-field system. This provides an indicator for attraction and/or trapping efficiency under the different experimental conditions.

Table 2: Summary of statistical model outputs was done separately for preliminary choice experiments in the absence of an eave fabric and for the impact of transfluthrin pre-exposed mosquitoes in experiments with eave fabrics. For both, modelling was done firstly for relative trapping efficacy of trap 1 out of all trapped and secondly for overall efficiency of both traps out of all mosquitoes released.

			Mosquitoes recaptured in trap 1 out of all mosquitoes trapped			Mosquitoes recaptured with both traps out of all mosquitoes released		
	Trap 1	Trap 2	Estimated* mean percentage (%) in trap 1	Odds Ratio (95% CI)	p-value	Estimated* mean percentage (%) trapped	Odds Ratio (95% CI)	p-value
Preliminary experiments in absence of eave fabric								
2 equal trap choices (experiment 1)	Odour-baited	Odour-baited	51.4 (95% CI 45.9-56.9)	1		53.7 (95% CI 46.1-61.3)	1	
2 equal trap choices (experiment 2)	UV light	UV light	54.4 (95% CI 45.7-62.8)	1.13 (0.75-1.68)	0.564	21.9 (95% CI 16.2-29.0)	0.24 (0.15-0.39)	<0.001
2 trap types choice (experiment 3)	Odour-baited	UV light	45.8 (95% CI 37.6-54.3)	0.80 (0.54-1.18)	0.265	23.0 (95% CI 17.1-30.0)	0.26 (0.16-0.41)	<0.001
Pre-exposure experiment in presence of eave fabric								
2 trap choices, unexposed mosquitoes (experiment 4)	Odour-baited	UV light	60.6 (95% CI 48.1-71.8)	1		34.1(95% CI 24.3-45.5)	1	
2 trap choices, transfluthrin pre-exposed mosquitoes (experiment 5)	Odour-baited	UV light	57.0 (95% CI 47.6-66.0)	0.86 (0.45-1.64)	0.651	33.1(95% CI 25.0-41.9)	0.96 (0.53-1.74)	0.883

*based on statistical model

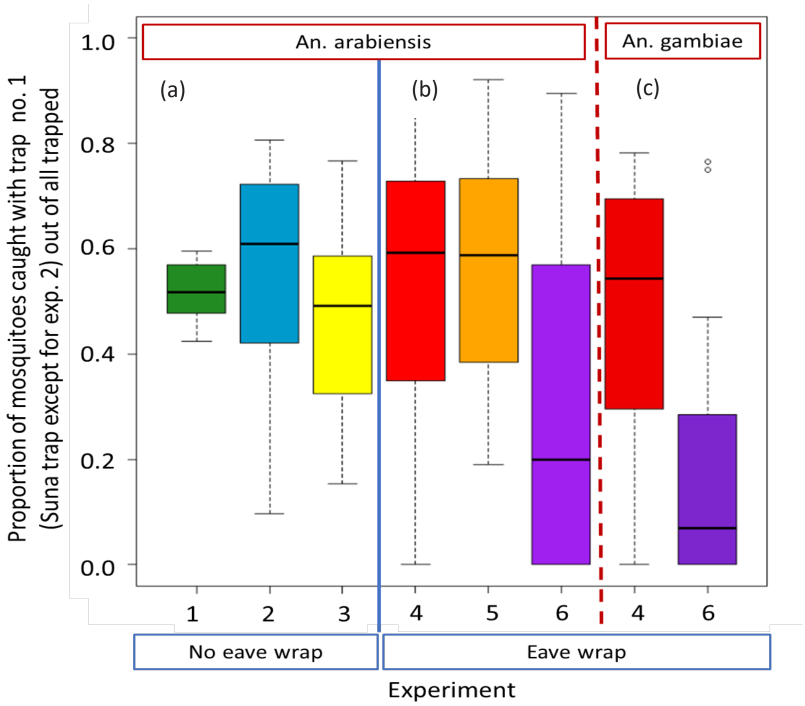

Figure 3A-C. Data exploration across the 6 different experiments (choice assays): The box plots illustrate the proportion of released *An. arabiensis* trapped in the reference trap no. 1 **out of all mosquitoes trapped**. In all but experiment 2, trap no. 1 was an odour-baited Suna trap. Experiments 1-3 were reference experiments providing two traps as choice in the absence of any treatment. Experiments 4-6 contained either untreated (experiments 4 and 5) or transfluthrin-treated fabric around the eave gaps of the hut; mosquitoes were either unexposed to transfluthrin before release (experiments 4 and 6) or pre-exposed (experiment 5). Different colours indicate different choice tests and experimental conditions (see Table 1).

Combined trap catches in the preliminary choice experiments were highest when two odour-baited Suna traps supplemented with CO_2 were set in the semi-field system as they recaptured around half of all mosquitoes released (Table 2; Figure 4A). When two CDC UV light traps, also supplemented with CO_2, were set up, the odds of trapping a released mosquito with two CDC UV light traps was 4 times lower than the odds when two Suna traps were used, with the collective recapture being only around a fifth of the mosquitoes released (Table 2; Figure 4A). A similar collective recapture rate was found when one Suna odour-baited trap and one CDC UV light trap were presented in the choice system (Table 2; Figure 4A).

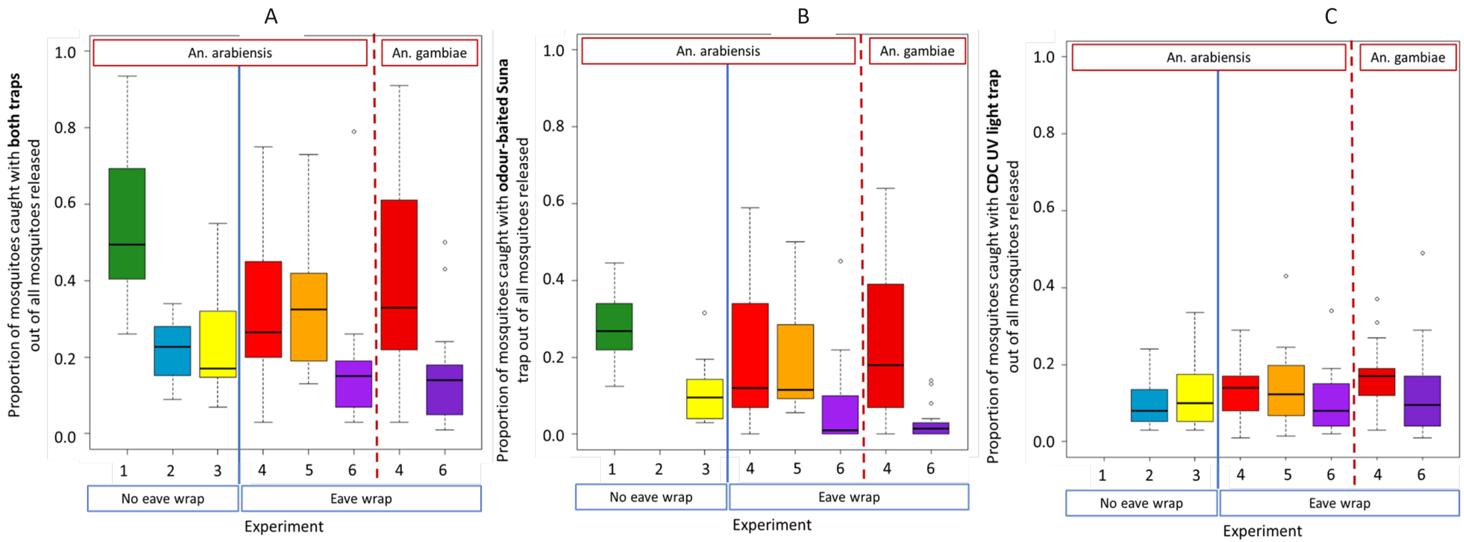

Figure 4. Data exploration across the 6 different experiments (choice assays): The box plots illustrate the proportion of released *An. arabiensis* trapped out of all mosquitoes released. (**A**) Proportion trapped with both traps. (**B**) Proportion trapped with odour-baited Suna trap. (**C**) Proportion trapped with CDC UV light trap. Experiments 1-3 were reference experiments providing two traps as choice in the absence of any treatment. Experiments 4-6 contained either untreated (experiments 4 and 5) or transfluthrin-treated fabric around the eave gaps of the hut and mosquitoes were either unexposed to transfluthrin before release (experiments 4 and 6) or pre-exposed (experiment 5). Different colours indicate different choice tests and experimental conditions (see Table 1).

Table 3: Summary of statistical model outputs seeking to investigate an association between the proportions of mosquitoes recaptured with traps and constant exposure of mosquitoes to transfluthrin.

Constant exposure experiment in presence of eave fabric			
	Estimated* mean percentage (%) of *An. gambiae s.s.* & *An. arabiensis*	**Odds Ratio (95% CI)**	**p-value**
Mosquitoes recaptured in *odour-baited Suna* trap out of all mosquitoes trapped			
Experiment			
Untreated eave fabric (experiment 4)	59.0 (95% CI 51.9-65.7)	1	
transfluthrin treated fabric (experiment 6)	29.7 (95% CI 20.9-40.4)	0.45 (0.22-0.92)	0.032
Species			
An. arabiensis		1	
An. gambiae		0.85 (0.49-1.49)	0.581
Experiment*Species interaction			
transfluthrin treated fabric * *An. arabiensis*		1	
transfluthrin treated fabric * *An. gambiae*		0.39 (0.13-1.20)	0.105
Mosquitoes recaptured with *odour-baited Suna* trap out of all mosquitoes released			
Experiment			
Untreated eave fabric (experiment 4)	21.6 (95% CI 16.1-28.1)	1	
transfluthrin treated fabric (experiment 6)	4.94 (95% CI 2.56-9.32)	0.29 (0.12-0.73)	0.011
Species			
An. arabiensis		1	
An. gambiae		1.10 (0.57-2.17)	0.767
Experiment*Species interaction			
transfluthrin treated fabric * *An. arabiensis*		1	
transfluthrin treated fabric * *An. gambiae*		0.36 (0.08-1.70)	0.201
Mosquitoes recaptured with *UV light* trap out of all mosquitoes released			
Experiment			
Untreated eave fabric (experiment 4)	15.2 (95% CI 12.2-18.9)	1	
transfluthrin treated fabric (experiment 6)	11.4 (95% CI 8.77-14.8)	0.72 (0.49-1.06)	0.098
Species			
An. arabiensis		1	
An. gambiae		1.30 (0.89-1.91)	0.178
Experiment*Species interaction			
transfluthrin treated fabric * *An. gambiae*		was not retained in final model	0.995
*based on statistical model			

The percentages of mosquitoes trapped with individual CDC UV light traps out of all released mosquitoes were similar (p=0.342) when set in the presence of another light trap (10%; 95% CI 7-14%) and when set in the presence of an odour-baited Suna trap (12.5%; 95% CI 9.1-16.8%; Figure 4C). Individual odour-baited traps caught around 3 times more mosquitoes (OR 3.2 (95% CI 1.8-5.7), $p < 0.001$) when set up in the presence of another odour-baited trap (27.7% (95% CI 21.6-34.6%) than when set up in presence of a CDC UV light trap (10.5% (95% CI 6.8-15.9%); Figure 4B).

Investigating the effect of transfluthrin on trapping (experiments 4-6; presence of eave wrap)

Transfluthrin pre-exposed *An. arabiensis*

Pre-exposure was neither associated with the ratio with which the mosquitoes were collected with the odour-baited Suna trap (Figure 3, comparing experiments 4 and 5; Table 2) nor with the overall recapture rate out of all released mosquitoes (Figure 4, comparing experiments 4 and 5; Table 2).

Continuous transfluthrin exposure of *An. arabiensis* and *An. gambiae s.s*.

Continuous exposure to transfluthrin-treated eave fabric in experiment 6 was associated with a reduction in proportional catches of host-seeking *Anopheles* in the Suna trap (out of all trapped; Figure 3, Table 2). The analysis did not suggest a statistically significant main effect of a species-specific trapping efficacy, but the interaction term between the treatment and mosquito species implies that *An. gambiae s.s.* might be more affected by the transfluthrin than *An. arabiensis* and consequently less likely to be trapped by the odour-baited trap in the presence of transfluthrin (OR 0.39; Figure3 and 4, Table 3) even though this did not reach statistical significance.

A reduction in proportional catches of host-seeking *Anopheles* in the Suna trap was also observed when the efficiency in recapturing mosquitoes out of all released was examined. Analysing the outcome for the two traps separately revealed that the odds of trapping a mosquito in the Suna trap (out of all released) when constantly exposed to transfluthrin was over 3 times lower (OR 0.29) than when unexposed (Table 3). Again, a species-specific interaction suggests that the trapping efficiency for *An. gambiae s.s.*was more affected than for *An. arabiensis* (OR 0.36; Table 3). The trapping efficiency of the CDC UV light trap was more or less consistent under most experimental conditions (Figure 4) with a trend of slightly reduced trapping (OR 0.79) with the CDC UV light trap in presence of a Suna trap and the spatial repellent. This did not reach statistical significance (p = 0.098) and the minimal impact left this trap still superior to the odour-baited trap. The analysis suggests that *An. gambiae s.s.* might be slightly more responsive to UV light traps than *An. arabiensis* (OR 1.30) in general, irrespective of the presence or absence of transfluthrin; however, again this did not reach statistical significance.

Discussion

In the present study, the trapping efficiency of an odour-baited Suna trap was greatly impacted by the presence of transfluthrin in the semi-field system. In the absence of the repellent, one odour-baited trap caught more than a quarter of all the mosquitoes released. This reduced to just below 5% of the total released when the repellent was present. Momentary exposure of experimental mosquitoes to the repellent prior to release did not affect the trapping efficiency of the odour-baited trap. Interestingly, the trapping efficiency of an odour-baited trap was also influenced by the presence of either a second odour-baited trap in the system or the presence of a light trap. When two odour-baited traps were in a system, their combined trapping efficiency was highest in this particular experiment as they were able to recapture more than half of the total released mosquitoes (Hiscox *et al.*, 2014), while when set up in combination with a UV light trap, the trapping efficiency of an odour-baited trap reduced to an eighth of the total released when no repellent was included in the system. In comparison, earlier experiments showed that a human being was able to trap more than 70% of the mosquitoes released in the semi field system as seen in previous work (unpublished data – Chapter 2).

The exact reasons why there was a disparity between recapture rate of the odour-baited trap in the presence of a second odour-baited trap compared to when in the presence of a light trap are unknown. One reason may be that the increased synthetic lure concentration in the semi-field system, working with CO_2 may have increased flight activation and attraction of mosquitoes to the traps, similar to host-seeking events (Takken *et al.*, 1997a; Dekker *et al.*, 2001; Dekker *et al.*, 2005; Dekker *et al.*, 2011). With this assumption, it would also be expected that odour-baited traps would have captured more mosquitoes than the light traps due to the presence of the synthetic lure in addition to CO_2 (Qiu *et al.*, 2007; Cardé *et al.*, 2010b; Cardé *et al.*, 2010a) and in the absence of any external interference. In the present study, the trapping efficiency of the odour-baited trap was greater than that of the CDC UV light trap except in the preliminary experiment 3; the calibration experiment done in the absence of any eave fabric, when the CDC UV light catches were slightly higher. This exception was unexpected and remains unexplained.

Trapping efficiency of the CDC UV light trap was interestingly not affected by the presence of the repellent. In fact, the proportion of recaptured mosquitoes remained relatively constant (10-12%) in the light trap; both in the absence and in the presence of the repellent. However, the proportion of mosquitoes trapped by the light traps was low relative to the total number of mosquitoes released. The reason for this low recapture rate could have been that light, even when supplemented with CO_2, may not provide clear directional and specific cues for host-seeking mosquitoes that make it a suitable attractive cue compared to the combination of CO_2 and odour (Platt *et al.*, 1957; Stark *et al.*, 1982; Takken *et al.*, 1997a). In spite of this, light remains a very important modulator of mosquito behaviour (González *et al.*, 2016; Hawkes *et al.*, 2016; Costa-Neta *et al.*, 2017; Ponlawat *et al.*, 2017a; Sheppard *et al.*, 2017) exemplified by how

exposure to white light or moonlight significantly affects the host-seeking behaviour of malaria mosquitoes (Bidlingmayer, 1964; Sheppard *et al.*, 2017).
Use of LED lamps that emitted blue light of 470 nm wavelength or green light of 520 nm wavelength was shown to catch high numbers of *Anopheles* mosquitoes in field assays compared to incandescent light (Costa-Neta *et al.*, 2017). Preference for UV (including near ultraviolet UV-A black light of 315-400 nm) and LED light over the standard CDC incandescent light by *Anopheles* mosquitoes was seen in a different study and on this basis UV light was selected over incandescent light in this study (Li *et al.*, 2015a; Mwanga *et al.*, 2019).

Species-specific behavioural responses to olfactory cues have been shown in previous studies and were attributed to differences in behaviour possibly due to divergent chemosensory receptor where one species may detect a repellent (e.g. *Aedes* and *Culex* to DEET) while another does not (*Anopheles* to DEET)(Ghaninia *et al.*, 2019; Afify *et al.*, 2020). During the development of the MB5 synthetic lure, attractive chemicals were assessed based on response to *An. gambiae s.s*. and it was only in later studies that responses by other malaria vectors to the synthetic blend were determined (Mukabana *et al.*, 2002; Olanga *et al.*, 2010; Verhulst *et al.*, 2011; Mukabana *et al.*, 2012a; Hiscox *et al.*, 2014; Menger *et al.*, 2014; Menger *et al.*, 2015; Homan *et al.*, 2016; Menger *et al.*, 2016; Mburu *et al.*, 2017; van de Straat *et al.*, 2019). The present study was able to show both similar and dissimilar responses of the two sibling species; *An. arabiensis* and *An. gambiae s.s.* Proportional catches of the sibling species in the odour-baited trap in the absence of the repellent were comparable. The repellent, however, seemed to adversely affect *An. gambiae s.s.* as a lower proportion was caught in the odour-baited trap when transfluthrin was present compared to *An. arabiensis* under the same experimental conditions. On the other hand, *An. gambiae s.s*. seemed more responsive to the UV light trap in general compared to *An. arabiensis*. The sibling species studied have previously been shown to not only have different host-preferences but to also respond differently to various cues, partially explained by the structural and genetic differences in their odorant-binding proteins (van den Broek *et al.*, 1999; Li *et al.*, 2004; Li *et al.*, 2005; Lorenz *et al.*, 2013; Ghaninia *et al.*, 2019).

The ideal mode of action for spatial repellents in mosquito-borne disease control has been highlighted as the ability to mask human host odours or at least induce an avoidance behaviour or feeding inhibition, therefore limiting the host-vector interaction (Schreck *et al.*, 1970; Achee *et al.*, 2009; Achee *et al.*, 2012; Ray, 2015). The repellent effect in general points to some influence in the perception and location of potential hosts through interference in odour-detection (Hao *et al.*, 2008; Turner *et al.*, 2011).

In our study, the reduction in proportional catches of mosquitoes in odour-baited traps in the presence of the repellent may be explained by possible interference with the perception of the lure. As all mosquitoes were released less than 1.5 m from the point of application of repellent and at least 3 m from either trap, the assumption that the repellent plume influenced the mosquito perception of odours is plausible. The use of an odour-baited trap, at least in the relatively close vicinity of the repellent, is therefore unsuitable in the push-pull approach and further placement of these traps could be considered outside the area of protection of the repellent. In Chapter 2, the positioning of odour traps at 5 m was not protective against outdoor-biting *An. arabiensis* (Chapter 2, this thesis). Testing varied distances such as 7.5 m, 10 m and even 15 m could provide information on which would be the most beneficial positioning. While exploration of distance from the repellent plume could be one consideration, positioning of the trap in relation to other references such as breeding habitats (Okumu *et al.*, 2010a).

Previous studies have shown that repellents such as geraniol, citral, eugenol and anisaldehyde affect *Ae. albopictus* mosquitoes after prolonged exposure (48 h) such that their host-seeking ability is greatly affected leading to reduced movement towards hosts and probing (Hao *et al.*, 2008). In natural conditions, mosquitoes would normally fly out of a repellent plume to better reorient with host cues before attempting another approach and may spend just enough time in the plume to affect their immediate but not their subsequent host-seeking behaviour (Schreck *et al.*, 1970; Barnard, 2005; Klun *et al.*, 2006; Grieco *et al.*, 2007; Moore *et al.*, 2007; Hao *et al.*, 2008; Bohbot *et al.*, 2010).

While the trapping efficiency of the UV light trap was unaffected by the repellent and was able to perform trap better than an odour baited trap in the present study, it would be highly unlikely to replicate such artificial conditions in the field especially with regards to mosquito proximity to the treated house prior to picking up the host cues and how the airflow would influence the availability of the plume and host cues (Achee *et al.*, 2012; Kawada *et al.*, 2014; Menger *et al.*, 2015). It would be important to note that the CDC light trap was originally intended for indoor use (Garrett-Jones *et al.*, 1975), though recent studies have explored its utilization outdoors (Sriwichai *et al.*, 2015; Degefa *et al.*, 2017; Degefa *et al.*, 2020). Future studies could substantiate the differences seen in natural conditions and provide evidence on the suitability or otherwise of CDC UV light trap as a pull component. As a way forward, a more attractive pull trap, preferably an odour-baited one supplemented with additional host cues such as heat and humidity (Hawkes *et al.*, 2017) could be explored preferably in natural conditions. The reason for this is that the trapping efficiency of the odour-trap remains superior to that of the light trap in the absence of a repellent (Hiscox *et al.*, 2014; Mburu *et al.*, 2019). By adjusting the position of an improved odour-trap trap proximal to the repellent plume such that the mosquito encounters the trap prior to the repellent, the push-pull set up could be further improved (Okumu *et al.*, 2010a).

Conclusion

This study showed that MB5-baited traps are unsuitable in close vicinity to a spatial repellent for a push pull set up and that UV light traps might be more efficient and potentially easier to manage. However, since both traps types were not highly efficient even in the absence of a human, there is urgent need to develop more attractive traps by increasing the attractive cues (combining odours, warmth and humidity) that can be combined with spatial repellents in a push pull situation than currently available.

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Chapter 4

Less is more: Repellent-treated fabric strips as a substitute for full screening of open eave gaps for indoor and outdoor protection from malaria mosquitoes in Kenya

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To be submitted in a modified form

Chapter 4

Abstract

Providing protection from malaria mosquitoes, both indoors and outdoors, is crucial to curbing malaria parasite transmission. Screening of house entry points, especially with incorporated insecticides, confers significant protection but remains a costly and labour-intensive application. Use of spatial repellents has shown promise in creating areas of protection against mosquito bites in the peri-domestic area of a household. This study aimed at comparing the protection provided by transfluthrin-treated and untreated eave screens and at determining if an incomplete transfluthrin-treated eave strip would be a suitable replacement for a full screen. Human landing catches were implemented indoors and outdoors for separate experiments, with insectary-reared *Anopheles arabiensis* mosquitoes under controlled semi-field conditions.

The odds of a female mosquito finding a human volunteer indoors and attempting to bite was similar whether the eaves were completely open, or when there was an untreated fabric strip fixed around the eaves. The application of a transfluthrin-treated fabric strip achieved the same protection indoors (OR 0.07, 95% CI 0.04-0.13) as the treated full screen and was 4 times lower than the odds of receiving a bite in the presence of an untreated full screen (OR 0.30, 95% CI 0.20-0.47). The impact of transfluthrin treatment on outdoor-biting was correlated with evening temperatures during the experiments. At comparatively low evening temperatures, a transfluthrin-treated full screen provided moderate and variable protection from bites (OR 0.62, 95% CI 0.37-1.03), whilst at higher evening temperatures the odds of receiving a bite outdoors was over 4 times lower in the presence of transfluthrin, either on a full screen (OR 0.22 95% 0.12-0.38) or on a fabric strip (OR 0.25, 95% 0.15-0.42) than when no treatment was present (open eave as reference).

The findings suggest that transfluthrin-treated fabric strips can provide a substitute for complete eave screens. They are a simple, easy to handle tool for protecting people from malaria mosquito bites indoors and potentially around the house in climatic areas where evening and night-time temperatures are relatively high.

Keywords:

Transfluthrin, eave screens, eave strips, vector control, spatial repellent, human landing catches

Chapter 4

Introduction

Many malaria endemic areas in sub-Saharan Africa are rural, where housing structure pre-disposes people to mosquito bites; either because of open gaps along the eaves, uneven walls and unscreened windows and doors. House structures often necessitate evening activities, cooking for example, to be conducted outdoors or in structures near the main house that do not offer any protection from mosquito bites (Ghebreyesus *et al.*, 2000; Huho *et al.*, 2013; Wanzirah *et al.*, 2015; Kaindoa *et al.*, 2018).

Eave gaps provide ventilation indoors and sealing them off, while slightly protective against mosquitoes, can lead to reduced air flow and potentially increased indoor temperatures unless proper house adjustments are done to remedy this (Lindsay *et al.*, 2003; Jatta *et al.*, 2018). Improved housing structure has been shown to significantly reduce house-entry of adult mosquitoes and malaria infections (Lindsay *et al.*, 2002; Lindsay *et al.*, 2003; Kirby *et al.*, 2009; Tusting *et al.*, 2017; Corrêa *et al.*, 2019). Screening of open eave gaps in combination with screening of doors and windows is recommended for effective reduction of mosquito house entry(Kirby *et al.*, 2009).Despite the advantages of eave screening, a major challenge in this approach is the availability of low-cost materials, as well as the difficulty in properly fixing barriers in existing structures which may have uneven surfaces (Menger *et al.*, 2016; Ng'ang'a *et al.*, 2019). Furthermore, screening the house may reduce adherence to the use of long-lasting insecticide-treated bed nets (LLINs), due to perceived reductions in biting pressure (Ng'ang'a *et al.*, 2019) which could lessen the protection received by occupants.

In situations where house-improvement is not immediately achievable for varied reasons, and where LLINs provide incomplete protection against malaria infection, alternate means of vector control should be developed to provide protection to persons when they are in the periphery of their homes (outdoors), or indoors but not yet under protective bed nets (Mathania *et al.*, 2016).Evening and outdoor mosquito biting have been cited as contributors to the rising importance of residual malaria transmission that remains unaddressed (Durnez *et al.*, 2013; Killeen, 2014; Wamae *et al.*, 2015; Sherrard-Smith *et al.*, 2019).

Spatial repellents have received increasing attention in recent years as complementary control tools against adult malaria mosquitoes (Hao *et al.*, 2008; Achee *et al.*, 2012a; Ogoma *et al.*, 2012; Revay *et al.*, 2013; Andres *et al.*, 2015; Maia *et al.*, 2016; Norris *et al.*, 2017). Unlike toxicants that result in mortality of mosquitoes or irritants that result in agitation, spatial repellents drive mosquitoes away from a treated space, thereby being proposed as ideal candidates for outdoor application(Lynch *et al.*, 2016; Norris *et al.*, 2017). In addition, pyrethroid spatial repellents have been shown to induce deterrence, even in mosquitoes which are resistant to this group of insecticides (Agossa *et al.*, 2015; Lynch *et al.*, 2016). As a deterrent mode of action may not lead to complete toxic effects, the selection pressure exerted by spatial repellents might be lower than that exerted by toxicants which end up selecting resistant mosquitoes in the population by killing of the more susceptible ones(Birget *et al.*, 2015; Lynch *et al.*, 2016). There is, however, the unfortunate possibility of selection of multiple resistance genes which could occur gradually with constant use of deterrent insecticides leading to increasing low-level resistant mechanisms (Gressel, 2011; Guedes *et al.*, 2017). An advantage of spatial repellents is the possibility to exert repellency on a wide-range of mosquito species, therefore potentially disrupting not just the transmission of malaria, but other mosquito-borne diseases such as dengue, Chikungunya and Zika among others (Achee *et al.*, 2012a; Achee *et al.*, 2012b; Sathantriphop *et al.*, 2014). Additionally, spatial repellents can be highly effective against biting by nuisance mosquitoes.

One such candidate spatial repellent is transfluthrin, which is a fast-acting, low persistence, fifteen-carbon pyrethroid insecticide. It has been included in mosquito repellent and killing products such as mosquito coils and vaporizers(Morton *et al.*, 1947; Schreck *et al.*, 1970; Ramesh *et al.*, 2001; Nazimek *et al.*, 2011; Ogoma *et al.*, 2014b; Andres *et al.*, 2015).Release of transfluthrin on commercial products relies on an external source of energy or combustion and has many limitations: coils produce smoke and need to be actively managed for optimal protection(Hill *et al.*, 2014), electrical emanators are not affordable for most rural low-income communities and require a power socket in the area to be protected(Pates *et al.*, 2002). Non-powered, low maintenance passive release options would make this intervention more accessible for large-scale use (Pates *et al.*, 2002; Ogoma *et al.*, 2012; Masalu *et al.*, 2017).

Inclusion of the repellent on eave screening fabric for passive release could increase the protection conferred indoors by existing tools and could potentially allow peri-domestic release of the repellent thereby also providing the much-needed bite protection outdoors, even against pyrethroid-resistant malaria vectors (Kawada *et al.*, 2014; Andres *et al.*, 2015; Govella *et al.*, 2015; Bowman *et al.*, 2018; Deletre *et al.*, 2019). Recent work done in Tanzania has explored the use of insecticide-treated strips that are secured on traditional houses to achieve a protective effect against malaria mosquitoes instead of the use of full eave screening (Ogoma *et al.*, 2012; Govella *et al.*, 2015). The idea of using a repellent-treated fabric that does not fully cover the eave gaps offers possible cost and logistical advantages as there would probably be less material used and less precise fitting necessary as compared to the treated or untreated-eave screen (Kirby *et al.*, 2009; Ng'ang'a *et al.*, 2019).

Chapter 4

The present study aimed at comparing the protection provided by both treated and untreated full screening, and incomplete screening with treated and untreated eave strips against bites from insectary-reared *Anopheles arabiensis*(Mbita strain, Kenya) under controlled semi-field conditions. Human landing rates, both indoors and outdoors, were measured as a basis for determining if a transfluthrin-treated eave strip would be a suitable replacement for a full eave screen (treated or untreated).

Materials and methods

All experiments were conducted under semi-field conditions at the International Centre of Insect Physiology and Ecology at the Thomas Odhiambo Campus (icipe-TOC) in Mbita, on the shores of Lake Victoria in Homabay County, western Kenya (0°26'06.19"S, 34°12'53.13"E; altitude 1,137 m).

Semi-field systems

Two mesh-screened greenhouses (Amiran Kenya Ltd, Nairobi, Kenya) measuring 27 m long, 11 m wide and 4.3 m at the highest point were used for experiments. These systems were made of steel-structured frames with a Solarig^{TM} covered roofs and 17-mesh netting (17 apertures per every linear inch of mesh) on all sides to ensure adequate ventilation (Figure 1). The floor of the semi-field systems was covered with up to 30 inches of sand and was kept clear of any vegetation. The sandy floors were watered daily prior to experiments to ensure that the relative humidity remained above 70%. Temperatures inside the semi-field system varied during the months the experiments were implemented (September 2018 to March 2019) between a minimum of 18 °C at night and a maximum of 50 °C during the day. Data loggers (Tinytag View 2 Data Logger, Gemini data loggers, UK) were placed in both semi-field systems and temperature readings were taken every 30 minutes throughout. Each semi-field system contained a make-shift hut made of angle iron frames, ply wood walls and grass-thatched gable roofs with open eave gaps to mimic a traditional house in western Kenya. Each traditional house measured 6.5 m by 3.5 m and 2.5 m at the highest point (Figure 1). Between the roof and the top of the wall, all round the house was a 0.1 m wide eave gap that was representative of gaps left open in traditional western Kenyan houses. The doors and windows of the experimental huts were fully screened. Human landing catches (HLC) were conducted either outdoors 2.5 m away from the experimental hut or inside at the centre of the hut.

Figure 1. One of the semi-field systems where the bioassays took place. Inside each semi-field system, that measures 27 m by 11 m, there was an experimental house made of plywood with a grass thatched roof and the frames made of angle iron to mimic a traditional western Kenya house. The experimental huts measured 6.5 m by 3.5 m.

Mosquitoes

Host-seeking female *Anopheles arabiensis* Mbita strain were obtained from the icipe-TOC mosquito insectary and were used for all bioassays. Rearing of mosquitoes in the insectary followed a standard procedure (Mbare *et al.*, 2013) where they were reared under ambient conditions. Female mosquitoes, between 3 and 5 days old post-emergence that had never fed on blood were selected for use in these experiments. Selection was done by holding a hand close to the outside of the cage and picking responding females out of the cage with a mouth aspirator. In each experimental night, 160host-seeking female mosquitoes were used per semi-field system. Selected mosquitoes were starved of water and glucose for 3-5 h prior to release in the semi-field systems. In each semi-field system, 40 mosquitoes were released from cups placed in all 4 corners. Mosquitoes were dusted using a distinct fluorescent dye to distinguish them according to the four sites of release(Verhulst *et al.*, 2013).

Insecticide susceptibility

World Health Organization (WHO) insecticide susceptibility cone tests were conducted on the insectary-reared *An. arabiensis* females (WHO, 2013). Four cones were used to test susceptibility of mosquitoes to 0.05% deltamethrin by exposing mosquitoes to treated papers for one hour and then monitoring them for 24 h. Knock down and mortality were compared to a control group that was exposed to untreated paper in parallel. In all susceptibility tests, between 20 and 25 mosquitoes were used in each cone with four cones being used to expose mosquitoes to test paper and two cones to expose mosquitoes to the castor oil-treated control paper (WHO, 2013).

Preparation of eave screens and strips

For full eave screens, burlap material from local markets was cut into bands measuring 21m by 0.12m to ensure full coverage of the eave gaps when fixed. For incomplete screening, narrow fabric strips were cut at the same lengths but only at 0.05m wide (Figure 2).

Figure 2: Eave screen secured on the eave gap of the experimental hut using aluminium wires to ensure equal complete coverage of the eave gap by the eave fabric leaving no gap above or below.

Emulsified transfluthrin from Bayer Global (Leverkusen, Germany) supplied at a concentration of 0.2 g/ml was used as a spatial repellent. As transfluthrin is insoluble in water (WHO, 2006; Ogoma *et al.*, 2012), the emulsified concentrate simplified the preparation of a solution and the impregnation of fabric (Ogoma *et al.*, 2012).

For treatment, a transfluthrin solution was prepared with approximately 1300 ml of water for each hessian screen and 700 ml for each hessian strip to ensure the fabrics are fully wetted without any dripping of the solution. Emulsified transfluthrin was added to the water to achieve 2.5 g active ingredient per m^2 of fabric. Eave screens and strips were kneaded into the transfluthrin solution until visibly saturated (Ogoma *et al.*, 2012). All fabric was dried in a non-experimental screened hut overnight immediately after impregnation, then rolled up carefully and stored, wrapped in aluminium foil, in a dark room at room temperature. Untreated screens or strips were soaked in plain water and then dried, and used in the same way as the treated fabric but stored at a different location.

During experiments, fabric was retrieved from the cold room and put up on the experimental hut on day one in the evening then removed the next morning before being stored again in the cold room. The treated fabric was unwrapped and put along the eaves of the experimental hut every evening at 17.30 h. Care was taken to avoid contact with the walls of the hut when placing the fabric. At the end of every experimental night, the fabric was removed in the morning, rolled up, covered in aluminium and stored in a dark room at room temperature till evening when it was placed again. The same fabric was used for 16 experimental nights – the duration of one full experiment with a fresh set of fabric being used for every subsequent experiment. Untreated fabric was handled with a clean set of gloves to avoid cross contamination. For the application of eave screens, care was taken to ensure that the entire eave gap was covered each evening by using wires to secure the fabric over it (Figure 2). For the incomplete screen, the fabric strips were positioned at the centre of the eave gap, ensuring an equal amount of space left open both above and below the fabric (Figure 3).

Experimental procedure

Five experimental treatments consisting of: (i) no eave fabric (open eaves), (ii) untreated full eave screen, (iii) untreated strip, (iv) transfluthrin-treated full eave screen, and (v) transfluthrin-treated strip, were tested in six blocks of experiments as outlined in Table 1. Due to availability of two screen-house systems, two experiments were run at the same time (experimental block).Human landing catches (HLC) were conducted for four hours between 19:00 h and 23:00 h in all experiments for 16 nights.

Figure 3. Eave strip secured on the eave gap of the experimental hut using aluminium wires to ensure equal gap (2.5cm) left above and below the eave fabric.

Treatments were crossed-over between semi-field systems after every four days and a resting period of three days in-between to allow for aeration of experimental residues (Ogoma *et al.*, 2014a).

Four male volunteers (aged between 18 and 50 yr) were rotated between the semi-field systems and experimental nights to correct for human bias. The impact of the treatments was estimated separately for outdoor and indoor biting. For the simulation of outdoor biting, the volunteers sat on a chair situated 2.5 m away from the experimental hut, approximately in the middle of the semi-field system (semi-field system as shown in Chapter 2). For estimates of indoor-biting, the volunteers sat in the middle of the experimental hut. The volunteers were screened weekly for malaria parasite infections to prevent any circulation of infected insects in the semi-field systems and were exempted from participation till a negative result was confirmed. In preparation for the experiments, each volunteer cleaned their feet with odourless soap up to the knee and took position on the chair. Host-seeking *An. arabiensis* females were manually aspirated as soon as they landed on their lower legs of the volunteers. The mosquitoes were transferred to collection cups labelled with the hour of collection and semi-field system identification letter. Protective jackets to cover heads and arms were provided while torches were used for visualization when aspirating. Volunteers conducted HLC while shoeless with landings on feet included in the experimental outcome.

Table 1: Summary of all experimental procedures implemented as blocks of two test treatments in parallel in two semi-field systems. Indoor and outdoor human landing catches were done in independent experiments.

Experimental blocks	Location of human landing catches	Treatment in semi-field system A*	Treatment in semi-field system B*
1	Indoors	Open eave	Untreated incomplete eave strip
2	Indoors	Untreated full eave screen	Transfluthrin-treated full eave screen
3	Indoors	Transfluthrin-treated full eave screen	Untreated incomplete eave strip
4	Outdoors	Open eave	Untreated incomplete eave strip
5	Outdoors	Untreated full eave screen	Transfluthrin-treated full eave screen
6	Outdoors	Transfluthrin-treated full eave screen	Untreated incomplete eave strip

*All treatments were switched between semi-field systems every four days ensuring that in each experimental block, the treatments were equally applied in system A and B.

Data analysis

All analyses were carried out using R Studio statistical software from R core group version i386 3.5.1 (R Core Team, 2018). Associations between the proportion of mosquitoes landing on volunteers (number of mosquitoes collected out of all mosquitoes released) and test treatments were analysed using generalized estimating equations (GEE) fitted with a binomial distribution with logit link function. An exchangeable correlation matrix was assumed. The unique ID of every experimental night was included in the model as repeated measure. The experimental test and the volunteer ID were included as the fixed factors in the models. The experiment without any eave fabric in the system (open eave) served as reference. The model generated odd ratios (OR) and their associated confidence intervals (CI) which are reported. Mean proportions and their 95% CIs were estimated based on the model by transforming the log odds (logit) of the outcome to the odds scale and from the odds scale to the probability scale. The denominator in all experiments was the total number of females released per experimental night less the mortalities prior to release. Results from experimental nights where mortality in the release cups exceeded 10% were excluded from the analysis. The point of mosquito release had no significant association with the outcome and was removed from the final models. The possible correlation between the mean air temperature (in °C) during the four-hour mosquito collections duration of every experiment and the human biting rate was explored for transfluthrin containing experiments and for non-insecticidal experiments using the Pearson Correlation.

Ethical considerations

This study was approved by the Kenya Medical Research Institutes Scientific and Ethics Review committee (KEMRI-SERU), protocol number NON-KEMRI 546.

Results

Insecticide susceptibility test

In total, 88 *An. arabiensis* were exposed to 0.05% deltamethrin and 48 exposed to the untreated control (castor oil) in the WHO cone assay. Twenty-four hours after a 1 h exposure, a mortality of 93.2% was found, which was corrected to 91.15% according to WHO guidelines. This suggests that the insectary-reared mosquitoes used in consequent experiments were not fully susceptible to pyrethroid insecticides.

Indoor impact of treatments

In the absence of any fabric on the open eave gaps of the experimental huts, on average 45% (95% CI 38-52%) of all released mosquitoes were collected whilst seeking to bite the human volunteer indoors. This was similar when an untreated strip was placed at the eave gaps (Table 2, Figure 4). An untreated full screen had a highly significant impact on the proportion of mosquitoes seeking out the volunteer indoors (OR 0.3; Table 2); it prevented around half the bites a volunteer would have received in the absence of a screen (Table 2, Figure 4).

Treating the full screen with transfluthrin had a significant added benefit in reducing the proportion of mosquitoes seeking the host indoors. The odds of a mosquito landing on a human volunteer was 97% lower (OR 0.03) than it was when the screen was untreated based on the data from the experimental block no. 2 (Table 2, Figure 4).

Table 2: Association between proportion of mosquitoes landing on human volunteer and test treatments.

	Estimated* mean proportion of released *An. arabiensis* biting (95% confidence interval)	Odds Ratio (95% confidence interval)	p-value
	INDOORS		
Treatment			
open eave	0.45 (0.38-0.52)	1	
full untreated eave screen	0.20 (0.15-0.26)	0.30 (0.20-0.47)	<0.001
untreated eave strip	0.43 (0.36-0.50)	0.90 (0.66-1.24)	0.520
treated full eave screen (block 2)	0.03 (0.01-0.06)	0.03 (0.02-0.07)	<0.001
treated full eave screen (block 3)	0.06 (0.04-0.10)	0.08 (0.04-0.16)	<0.001
treated eave strip	0.05 (0.03-0.09)	0.07 (0.04-0.13)	<0.001
Volunteer			
no. 1	-	1	
no. 2	-	1.06 (0.73-1.55)	0.720
no. 3	-	1.03 (0.71-1.52)	0.820
no. 4	-	0.97 (0.53-1.40)	0.910
	OUTDOORS		
Treatment			
open eave	0.54 (0.45-0.63)	1	
full untreated eave screen	0.50 (0.41-0.60)	0.85 (0.53-1.41)	0.354
Untreated eave strip	0.57 (0.48-0.65)	1.14 (0.86-1.52)	0.547
treated full eave screen (block 5)	0.42 (0.33-0.51)	0.62 (0.37-1.03)	0.064
treated full eave screen (block 6)	0.19 (0.13-0.28)	0.22 (0.12-0.38)	<0.001
treated eave strip	0.21 (0.14-0.30)	0.25 (0.15-0.42)	<0.001
Volunteer			
no. 1	-	1	
no. 2	-	1.33 (0.83-2.13)	0.230
no. 3	-	0.90 (0.65-1.26)	0.592
no. 4		0.88 (0.68-1.13)	0.336

*based on statistical model

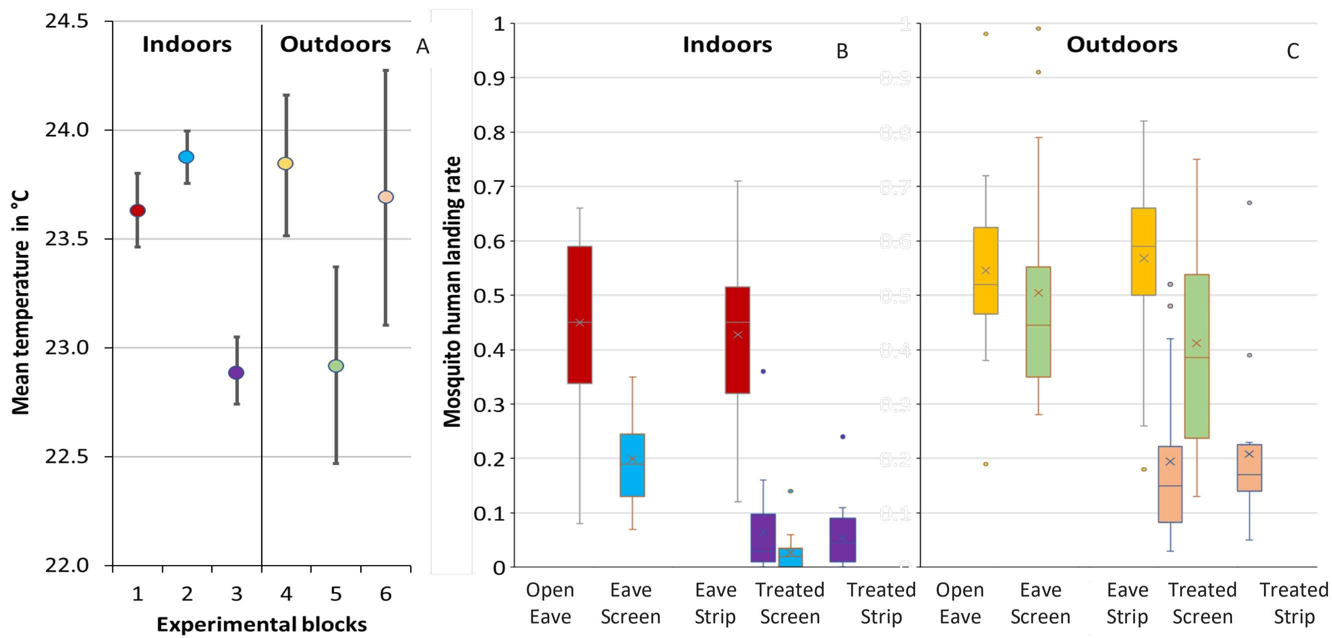

Figure 4. Graphs indicating A: mean temperatures across all experimental blocks, B: human landing catches (HLC) obtained indoors in the presence of the indicated conditions and C: HLC obtained outdoors in the indicated conditions.

Treated full screens were also tested in experimental block no. 3 and the odds of receiving a bite, even though still highly protective, was slightly higher (OR 0.08, Table 2) than in block no. 2. One difference observed between these two experimental blocks was a drop in the mean air temperature by around 1 °C (Figure 4) during the duration of the experiments. When comparing the impact of treated eave screens with treated fabric strips in experimental block no. 3, both were equally effective in reducing the odds of a mosquito approaching the volunteer indoors (Table 2).

Outdoor impact of treatments

The proportion of host-seeking mosquitoes recovered outdoors through human landing catches was with an average of 54% (95% CI 45-63%) around 10% higher than what was recovered indoors in the absence of any treatments.

No fabric (open eaves), untreated full screen and untreated fabric strips at the eaves led to the collection of similar proportions of released mosquitoes outdoors when landing on the human volunteers (Table 2). More clearly than for the indoor environment, the outdoor protection from transfluthrin as a spatial repellent appeared to be temperature dependent. In the experimental block no. 5 where the experiment with untreated full screen was run in parallel to the experiment with treated full screen, only a slight, borderline significant, reduction in human landing was observed, from an average of 54% of released mosquitoes landing on a volunteer in the absence of transfluthrin (open eave) to an average of 42% in the presence of transfluthrin ($p = 0.064$; Table 2). In contrast, when the treated full screen experiment was repeated in experimental block no. 6, only around 19% of the released mosquitoes landed on the human volunteer, presenting a statistically highly significant reduction ($p < 0.001$) when compared to no intervention and when compared to untreated full screen (Table 2, Figure 4). Mean temperatures between the two experimental blocks differed by around 1 °C (Figure 4). Pooling all data for experiments with transfluthrin and for experiments without transfluthrin in the system and performing a descriptive analysis found a negligible and statistically non-significant positive correlation between biting rates and air temperatures during the experiments ($r=0.186$, $p=0.099$) in the absence of transfluthrin. In contrast, in the presence of transfluthrin either on screens or strips, there was a negative association between landing rate and evening temperature ($r=-0.529$, $p<0.001$) with higher temperatures resulting in lower landing rates hence higher protection (Figure 5).

The impact of a treated screen and a treated strip was, as observed indoors, similar outdoors in reducing the proportion of host-seeking mosquitoes landing on human volunteers at the test conditions (mean temperature of 23.7°C; Figure 4). It must however be expected that a treated strip would equally not provide large reductions if evening/night-time temperatures are low as seen for treated full screen experimental in block 5.

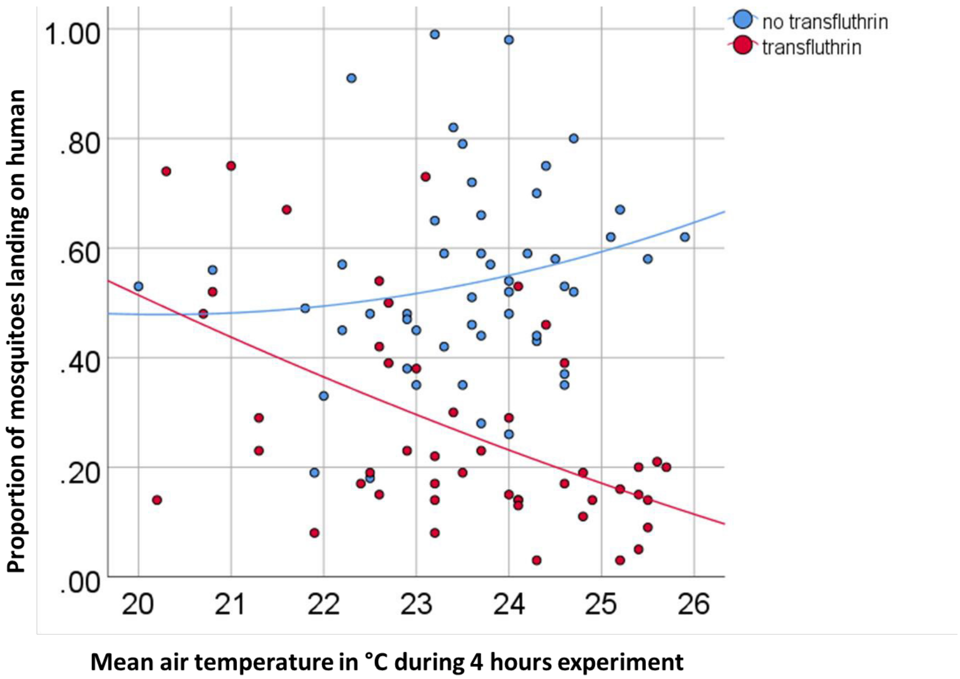

Figure 5. Scatter plot and trendlines indicating average air temperatures during the experimental runs with trendlines indicating how proportional mosquito recapture relate to temperature both in the presence and absence of transfluthrin.

Discussion

A strip of eave fabric treated with transfluthrin as a spatial repellent provided similar protection against both indoor and outdoor host-seeking mosquitoes compared with the treated screen, showing the plausibility of using a treated eave strip as a substitute to the treated eave screen. Untreated full screening was protective indoors but not outdoors, likely due to the physical barrier presented that prevented house-entry of mosquitoes. Treating a full eave screen with a spatial repellent added significant additional protection indoors compared to the reference group (open eaves) as well as the untreated eave screen. Treatment of eave screening could be envisaged in a scenario where screens are fitted to a house that has irregular walls and where it is not possible to seal all gaps and entry points. A repellent-treated full eave screen and a repellent-treated strip provided up to 60% protection to a human seated outdoors, at least within an area of around 3 m from the house outside, which we refer to as the peri domestic space.

Use of transfluthrin seemed to be greatly affected by the ambient temperature as lower temperatures during this experiment were associated with lower protection conferred outdoors by the treated full eave screen. It is assumed that the same effect would be observed for the treated strip, but during the experiments with the treated strip, mean temperatures were higher. Interestingly though, indoor protection was not affected by temperature, possibly due to close contact with house-entering mosquitoes, or already higher concentration of the repellent contained indoors that continued to confer protection even when temperatures reduced. This could also be attributed to higher temperatures indoors than those experienced outdoors – though it is important to note that the position of the repellent-treated eave screen was the same during indoor and outdoor HLCs. The impact of indoor temperature was however not tested as all temperature measurements were taken outdoors. Further experiments with treated full eave screening and treated strips, with temperature recordings taken indoors and outdoors across seasons could provide more information about the critical temperature threshold for transfluthrin used as a spatial repellent(Glunt *et al.*, 2014; De Keyser *et al.*, 2017; Ogoma *et al.*, 2017). From the initial studies in the absence of any treatment, indoor-biting rates of *An. arabiensis* were lower than those outdoors. The reason for this could partly be that following release of mosquitoes outdoors, they had easier access to the persons outdoors than to volunteers indoors who were partially protected by virtue of being inside a house which was a physical barrier. The mosquitoes gained entry through the open eaves as both the doors and windows were screened. The preferential outdoor-biting behaviour of *An. arabiensis* may also have contributed to the higher outdoor catches compared to indoors. While the presence of screens; both treated and untreated, significantly reduced the number of mosquitoes indoors, there were still mosquitoes that managed to gain entry, possibly through insignificant gaps such as the grass-thatched roof. This substantiates the need to continue using current vector control tools even as supplementary tools are developed.

The role that house structure plays in either aiding or preventing mosquitoes in gaining entry has been elaborated in many studies (Ghebreyesus *et al.*, 2000; Lindsay *et al.*, 2002; Njie *et al.*, 2009; Wanzirah *et al.*, 2015). This has led to several suggestions of ways to improve house designs in order to reduce the vector-host contact towards malaria control (Tusting *et al.*, 2017). One of the major improvements that have been proposed is screening of the eave gap that is usually left open in rural houses for both ventilation and structural purposes (Ghebreyesus *et al.*, 2000; Lindsay *et al.*, 2002). While in the long run the improvement of house design would be desirable for all, it may not be immediately achievable due to the financial handicap that plagues many people in malaria endemic areas (Tusting *et al.*, 2017). Development of tools such as the ones investigated in the current study that work on current house designs should be prioritized to protect people from receiving potentially infective bites. The application of treated eave strips could be a simple modification to houses, which does not require complex technical knowledge, or permanent changes to the house structure.

In line with previous studies, the current study was able to confirm that full eave screens are an effective way of creating a physical barrier against mosquitoes and keeping house entry rates low (Ogoma *et al.*, 2010; Menger *et al.*, 2016). Confirming the findings of Menger *et. al.* (2016), our results showed limited additional benefit of adding a spatial repellent to a full eave screen where indoor biting rates were the sole outcome measure. If eaves are screened fully, the physical barrier prevents house entry. However, one of the major practical and financial challenges of house screening is ensuring the availability of screens that cover open spaces adequately, particularly where variation in housing structures exists. For scale-up of full eave screening across whole areas, eave screens would need to be repeatedly adapted for each individual house to ensure that all gaps are sealed. This would be labour-intensive and add substantially to the cost of such an intervention.

To counter this inconvenience, the use of repellent-treated eave strips in place of eave screens was suggested. In our study, eave strips filled only half the width of the eave gap in our experimental huts. This meant that the physical barrier present with eave screens, whether treated or untreated, was absent in the eave strip. This lack of physical barrier with an eave strip and impact on house entry was indeed demonstrated in our results where the untreated eave strip did not offer protection indoors as entry of mosquitoes was not prevented.

Use of treated eave strips instead of treated full eave screens would be preferred for several reasons: less fabric would be needed, lower amount of insecticides would be used, a greater applicability to houses of different sizes of eave gaps would be possible and less expertise in ensuring sealing of eave gaps would be needed. The strips would be easier to install and to replace than a full eave screen. In our study, we used half the amount of fabric for eave strips as in eave screens, as well as half the amount of transfluthrin. For a wider eave gap, the reduction in amount of fabric required compared to full screening would be relatively greater, as we anticipate that the same narrow strip of fabric could be used. Variations of the width of eave wrap were not done in this study but could provide measurements of the extent of protection that can be conferred using a 0.05 m wide strip of fabric. We observed protection in outdoor landing rates at 2.5 m from the house, indicative of a substantial spatial repellent effect.

Previous studies have shown that anopheline mosquitoes enter the house predominately through open eaves (Snow, 1987). By sealing these eaves with physical barriers, even in the absence of any insecticide, house entry can be reduced considerably(Atieli *et al.*, 2009; Kampango *et al.*, 2013; Mburu *et al.*, 2018). During house entry, Spitzen *et. al*. 2016 showed that the majority of *An. gambiae* mosquitoes spent a considerable amount of time near the eave gap, proving that an intervention that exposes these mosquitoes to an insecticide or repellent at this point, even at sub-lethal concentrations, can have a significant impact on house entry (Spitzen *et al.*, 2016). If sub-lethal effects have an impact on host-seeking behaviour, this impact on house entry and outdoor biting, could extend to a wider-scale community-level effect. Further work, preferably at the field level, would be needed to demonstrate this conclusively.

In the present study, the use of transfluthrin on eave fabrics further confirmed the potential that spatial repellents have in diverting outdoor-biting mosquitoes that are currently not targeted by any other tool. It is however of particular concern that its protective effect is greatly affected by temperature conditions making future work on how to enable vaporisation of transfluthrin at lower temperatures critical (Glunt *et al.*, 2014; Ogoma *et al.*, 2017). It would be necessary to find ways to increase vaporisation without adapting costly technology with a reliance on electrical power (Masalu *et al.*, 2017). In spite of mortality rates of 91% to pyrethroids, that according to the WHO guidelines is indicative of presence of resistance, mosquitoes in the present study responded to transfluthrin showing promise of spatial repellent use even in times of rising pyrethroid-resistance(WHO, 2013).This advantage is, however, not a long-term guarantee as already studies have shown a reduction of repellent effect on resistant mosquitoes (Agossa *et al.*, 2015) and in the long run, alternative pyrethroid and non-pyrethroid spatial repellents should be considered.

From the present study, while availability of the hessian fabric meant ease of access to the quantity required, the relatively short lifespan of the impregnated repellent may be a challenge if rollout would be considered. In previous studies, repellent on hessian fabric was shown to be effective for up to six months (Ogoma *et al.*, 2012). In our studies, while the exact lifespan of the fabric was not measured, gradual reduction in efficacy was noticed in baseline studies (data not reported here), leading to a decision to use each fabric for a maximum of one experiment spanning sixteen experimental days. If the efficacy were found to decline over a span of more than a month, then the need to replace or re-treat the fabric could increase the cost of the tool making it less ideal for application in rural communities (Kirby *et al.*, 2008; Masalu *et al.*, 2017).

The impregnation process itself, while relatively easy, involved handling of 0.2g/ml concentration of transfluthrin. While this concentration was below the amount considered risky, handling would require semi-skilled personnel for safety purposes(Tisch *et al.*, 2005). Treatment and handling of the eave fabric in future studies needs to be re-thought in terms of how to increase safety for the people involved in preparing them.

Looking ahead, the use of hessian fabric holds promise as the material is relatively durable, available and mostly affordable (Ogoma *et al.*, 2012; Govella *et al.*, 2015; Masalu *et al.*, 2017). Sourcing for alternative material that may be more available and could remain treated for even longer than hessian fabric, if not longer, may be some of the next steps to be considered. Currently, plastic shields impregnated with transfluthrin have already shown promise due to their relative longevity, easier to handle as they come pre-treated and their ease of application in spaces where protection needs to be achieved(McPhatter *et al.*, 2017).

Malaria transmission is not limited to indoor-biting mosquitoes and as such, tools that can target outdoor-biting mosquitoes are needed to further supplement the existing tools(Killeen, 2014; Sangoro *et al.*, 2014; Benelli *et al.*, 2017). Our results confirm similar experiments implemented in Tanzania in parallel to the here presented work (Mmbando *et al.*, 2018) and demonstrate that the inclusion of a spatial repellent on the screen or strip offers protection against *An. arabiensis* biting in the outdoor environment. Protection against other mosquito species that are inherently outdoor and indoor-biting can be explored to further improve the suitability of this tool for mosquito control.

Further studies could be done to establish the range of protection and potential community effect conferred to provide further information for scaling up of this promising new vector control tool while ensuring that issues arising such as those seen during incomplete coverage (Maia *et al.*, 2016) are adequately addressed, possibly by the addition of supplementary tools to work in complement such as attract and kill devices (Matowo *et al.*, 2013; Mafra-Neto *et al.*, 2019; Mbare *et al.*, 2019).

Conclusion

Transfluthrin-treated fabric strips can provide a simple, easy to handle tool for protecting people from malaria vector bites in the peri-domestic space both indoors and outdoors around the house in climatic areas where evening and night time temperatures are high. Field studies need to confirm these findings under more variable natural conditions and to determine the range and longevity of the spatial repellent effect.

Acknowledgements

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Chapter 5

Pre-evaluation of push-pull mosquito control under natural conditions in western Kenya

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To be submitted in modified form

Chapter 5

Abstract:

Residual malaria transmission is fuelled in part, by lack of vector control tools that target outdoor-biting mosquitoes. New vector control tools are needed to effectively protect susceptible human hosts from outdoor-biting malaria vectors. Transfluthrin, released passively from an eave wrap as the "push" component, and a Suna trap baited with the MB5 odour blend as a "pull" component were tested under natural conditions in western Kenya; both separately and combined as a "push-pull" tool on their effect on indoor and outdoor host-seeking mosquitoes. Human landing catches were conducted both indoors and outdoors with secondary data obtained from the Suna trap. In the push-pull treatment there were fewer primary malaria vectors *Anopheles gambiae* s.l. and *An. funestus* s.l. mosquitoes indoors than in the control by 4-fold and by 3-fold, respectively. The push only treatment had a similar effect on indoor mosquito numbers as the push-pull treatment. Outdoors, catches of host-seeking primary malaria vectors were not associated with any of the treatments applied. *Culex* spp. and *Mansonia* spp. catches outdoors were reduced both in the presence of push-pull and push only components. It is concluded that the push-pull system with transfluthrin and an odour-baited trap was effective in controlling indoor-biting malaria vectors and outdoor-biting non-malaria mosquitoes.

Keywords:

Residual malaria transmission, Suna trap, Mbita blend 5, Transfluthrin, Push-pull, Mosquito control

Introduction

Development of new and improved vector control tools remains a high priority to counter recent impediments in malaria control (WHO, 2019). Increased coverage of long-lasting insecticide treated bed nets (LLINs), as well as improved diagnostics and treatment contributed to the huge and significant reductions in malaria (Bhatt *et al.*, 2015), but since 2015, further declines have stalled prompting the need for the development of new tools (WHO, 2019). The increase in insecticide resistance and the growing importance of outdoor malaria transmission are concerns that should be addressed to enhance malaria control (Killeen, 2014; Mathania *et al.*, 2016; Meyers *et al.*, 2016; Benelli & Beier, 2017; Moshi *et al.*, 2017; Saavedra *et al.*, 2019; Sherrard-Smith *et al.*, 2019; WHO, 2019).

Development of the push-pull mosquito control tool was designed to target outdoor-biting malaria vectors and to reduce the number of house-entering mosquitoes (Moshi *et al.*, 2017; Saavedra *et al.*, 2019; Sherrard-Smith *et al.*, 2019). The etymology of the tool was derived from the actions exerted by the constituent components with the 'push' exerted by a potent spatial repellent and the 'pull' being applied by a trapping and killing mechanism that is baited with attractants. Extensive semi-field experiments were carried out that screened a diverse range of chemicals for each component of the 'push-pull' system

(Chapter 2, this thesis). In summary, para-menthane-3,8-diol (PMD) was not found to offer passive protection to susceptible humans outdoors against *Anopheles* mosquitoes possibly because of its characteristics as a contact repellent unless volatilized using electrical fans (Barasa *et al.*, 2002; Menger *et al.*, 2014; Murchie *et al.*, 2016). Transfluthrin, a pyrethroid with toxic and repellent effects on mosquitoes had previously been shown to confer a passive protective effect in reducing biting by *Anopheles arabiensis* mosquitoes on human hosts and had been included in some industrial repellent products (Pates *et al.*, 2002; Ogoma *et al.*, 2012; Ogoma *et al.*, 2014; Andres *et al.*, 2015; Govella *et al.*, 2015; Maia *et al.*, 2016; Masalu *et al.*, 2017; Ogoma *et al.*, 2017). Semi-field experiments determined that use of 2.5g/m^2transfluthrin impregnated in a strip of fabric applied around the eave as the “push” was effective to reduce outdoor human landing catches by close to 60%. This was therefore decided as the ‘push’ component in our tool (Chapter 2, this thesis).
The Mbita-blend (MB5), as odour bait released from Suna traps, had been shown to be attractive to Afro-tropical *Anopheles* mosquitoes, with field studies showing strong evidence of the impact of the traps on malaria vectors and malaria prevalence (Hiscox *et al.*, 2014; Hiscox *et al.*, 2016; Homan *et al.*, 2016; Menger *et al.*, 2016; Mburu *et al.*, 2019). Following extensive experiments, MB5 supplemented with 2-butanone was chosen as our “pull” component even though supplementation with CO_2 increased the catches of the trap in the absence of a human (Chapter 2, this thesis). For the reported field trial, supplementation of MB5 with CO_2 in the Suna trap was done.

The combined use of a transfluthrin spatial repellent (the push) and an odour-baited trap (the pull) on the human landing rate had to our knowledge not been tested in a natural setting. The present study aimed to evaluate the effect of push-pull mosquito control on outdoor and indoor-biting wild mosquito populations with transfluthrin as the spatial repellent. The impact of the separate push-pull constituents was tested as well to determine individual effects on both outdoor and indoor-biting mosquito populations with particular emphasis on malaria vectors.

Materials and methods

Field study site

The study was carried out at Kigoche village located in the Kano flood plains, western Kenya (00°34’S, 034°65’ E and 1158 m above sea level). In the flood plains, rice farming is undertaken as an agricultural activity and due to the standing irrigation water, mosquito breeding occurs all through the year. The study took place between June and August 2019. Annual rainfall in this area varies between 1000 and 1800 mm and follows a bimodal pattern with the long rainy season typically occurring between April and June while the short rains occur between October and November. Daily temperatures range between 17 – 31

°C while the average relative humidity is 65% (Mweresa *et al.*, 2015; Mburu *et al.*, 2017). The study was carried out immediately after the long rains prior to the start of the short rains with minimal rainfall recorded in the meteorology department.

Malaria transmission is perennial and caused primarily by *Plasmodium falciparum* which is transmitted by *An. arabiensis* and *An. funestus s.s.*(Mukabana *et al.*, 2012; Mweresa *et al.*, 2015).

Figure 1a. One of the study houses in Kigoche depicting iron sheets roofing, mud walls and wooden door and windows as well as a PVC tent under which the outdoor HLC were conducted. The solar panel on the roof was not linked to this particular study.

Figure 1b. Fitting of the hessian fabric strip in one of the study houses in Kigoche with the fastening of fabric done to ensure equal space above and below the fabric.

Study houses

Four houses each measuring 6 m by 4 m were recruited randomly from a cohort of houses used in a previous study (Menger *et al.*, 2015). All the houses were two-roomed, mud-walled with earthen floors and had corrugated iron-sheet roofs with open eaves and no ceiling (Figure 1a). During human landing catches (HLC) between 19.00 h and 23.00 h, the houses were occupied and householders continued their routine activities.

Transfluthrin eave wrap

Hessian (burlap) fabric procured from local markets was cut and sewn into strips measuring 21 m by 0.5 m and the edges secured with cotton fabric to prevent fraying. Emulsified transfluthrin from Bayer Global (Leverkusen, Germany) supplied at a concentration of 0.2 g/ml was used to impregnate the hessian fabric. Emulsification of transfluthrin eased the preparation of solution which is necessary for impregnation of fabric as transfluthrin is insoluble in water (Ogoma *et al.*, 2012).
The hessian strips were kneaded in a transfluthrin solution with 26.25 ml of EC added in approximately 1300 ml water to achieve a final impregnation concentration of 2.5 g/m^2 (Ogoma *et al.*, 2012). All fabric was dried under a shelter overnight immediately after impregnation and then rolled up, wrapped in aluminium foil and stored in a 4 °C cold room prior to transportation to the field site. Treated fabric was usually used within a minimum of two days and a maximum of one week after treatment. Fabric thus

treated was used consistently for up to one week (four experimental nights) before being removed and replaced three days later with a freshly treated fabric to allow for complete aeration of houses between treatments (Ogoma *et al.*, 2014).

Application of the eave strip was done by securing it along the eave gap of the houses using nails to fasten on the wooden wall pillars supporting the roof while ensuring an equal gap both above and below the material to allow air flow (Figure 1b). The eave gap was therefore not completely sealed using the transfluthrin-treated hessian fabric.

Odour-baited traps

Suna traps (Hiscox *et al.*, 2014) were used for all set ups and baited with MB5 nylon strips (Mukabana *et al.*, 2012; Mweresa *et al.*, 2015) supplemented with CO_2 which was produced nightly by the fermentation of molasses using yeast (Mweresa *et al.*, 2014). The traps were positioned directly next to the house near the bedroom window with the main trap entry positioned 30 cm from the ground as done in a previous study (Homan *et al.*, 2016). All traps were powered by 26 Ah/12V Chloride Exide batteries (Chloride Exide (K) Ltd, Kenya) which were charged once a week. The traps were run from 18:00 h to 06:00 h on experimental days.

Human landing catches

Human landing catches were conducted indoors and outdoors by four adult male volunteers living in the area and who had consented to participate in the study. Their ages ranged between 25 and 50 years. Weekly *Plasmodium* screening using SD Bio-line Rapid Diagnostic Test (Abbot Illinois, USA) was done on the volunteers and artemisinin combination therapy (ACT) administered in case of a positive test. Those who tested positive were excluded from the study for one week and replaced by one of two extra volunteers recruited as standby volunteers and also living in the same area. The volunteers washed their legs prior to the start of mosquito collection using non-odorous bar soap supplied by the investigators. The volunteers sat on chairs wearing appropriate protective clothing with only the legs exposed and, using a mouth aspirator, trapped mosquitoes landing on their exposed legs.

For indoor HLCs, a chair was positioned in the middle of the first room upon entering the front door designated ‘sitting room’ during which time normal household activities continued. Outdoor HLCs were conducted at 2.5 m from the house with a Polyvinyl Chloride (PVC) tent cover (Figure 1) provided as protection from rain. During outdoor HLC no CDC light trap was hung inside the house.

HLCs were conducted from 19.00 h to 23.00 h with up to 10 min break allowed every hour.

Mosquitoes caught were transferred to collecting cups labelled according to the collection hour (one collection cup per hour per volunteer) and stored in a cool dry place before being transported to the laboratory for identification and counting the next morning.

Mosquito identification

Morphological identification was done prior to enumeration of mosquitoes caught according to genus and species under the microscope (Gillies & Coetzee, 1987). All mosquitoes were thereafter stored in -20°C freezers for future studies. All collections were entered in an electronic data storage system.

Table 1: Treatment allocation to the four study houses during the 8-week study period following a Latin square design with HLC location indicated

	Week 1 (4 nights) Outdoor HLC	Week 2 (4 nights) Outdoor HLC	Week 3 (4 nights) Outdoor HLC	Week 4 (4 nights) Outdoor HLC	Week 1 (4 nights) Indoor HLC	Week 2 (4 nights) Indoor HLC	Week 3 (4 nights) Indoor HLC	Week 4 (4 nights) Indoor HLC
House 1	Push-pull	Pull only	Push only	Control	Push-pull	Pull only	Push only	Control
House 2	Pull only	Push only	Control	Push-pull	Pull only	Push only	Control	Push-pull
House 3	Push only	Control	Push-pull	Pull only	Push only	Control	Push-pull	Pull only
House 4	Control	Push-pull	Pull only	Push only	Control	Push-pull	Pull only	Push only

Study design

Each of the four houses was randomly allocated a number 1-4 and four treatments: push-pull, push only, pull only and control applied in each with a weekly variation according to Table 1.

The treatments in the houses were as follows:

- Push-pull house: Eave fabric, transfluthrin-treated; Suna trap, baited with MB5 + CO_2
- Pull only house: Eave fabric, not treated; Suna trap, baited with MB5+ CO_2
- Push only house: Eave fabric, transfluthrin-treated; Suna trap, unbaited
- Control house: Eave fabric, not treated; Suna trap, unbaited

After four nights all the fabrics and baits were removed and fresh ones were applied three days later to allow for sufficient aeration of the houses prior to the next treatment. Human landing catches were conducted for four weeks outdoors and then four weeks indoors in all the houses (i.e. all four out, then all four in). As Suna traps were part of the push-pull set up, catches in them were considered as secondary data when baited (pull only and push-pull) and when unbaited (push only and control).

Statistical analysis

All data analysis was done using R version 3.5.1 (R Core Team, 2018). Data preparation, visualization and statistical analyses were conducted using functions from the packages gee, geepack, effects and lme4. Generalized estimating equations (GEE) using the geeglm function were used to analyse the effect of different treatments; specifically the repellent only (push only), odour-trap only (pull only) and the combination (push-pull) against untreated control on mosquito catches(Ballinger, 2004). The GEE was fitted with a Poisson distribution and a log-link function with an exchangeable matrix.

Human landing catches (HLC) was the primary indicator to determine the effect of treatments on the number of host-seeking mosquitoes. Odour-baited Suna traps provided secondary indicators (pull only and push-pull).

Experimental days were included in the model as repeated measurements. Experimental houses and HLC volunteers were tested as factors and since they did not significantly affect the model, were subsequently excluded from the statistics. The treatment effect on both outdoor and indoor human landing rates (HLC) was calculated on the total number of female mosquitoes per species/genera per trapping night with a test on

Results

In total, 8267 mosquitoes were caught over the 8-week study in all the traps used: 1623 *Anopheles gambiae* s.l. among which, 1615 (99.5%) were female, 1159 *An. funestus* s.l. among which 1087 (93.8%) were female, 432 *An. coustani* s.l. among which 422 (97.7%) were female, 50 *An. pharoensis* among which 43 (86%) were female, 3893 *Culex* spp. among which 3883 (99.7%) were female and 1110 *Mansonia* among which 1089 (98.1%) were female.

All effects were calculated in relation to population of female mosquitoes.

House variations

The four houses had variations in mosquito catches though the differences were not statistically significant. The estimated mean catches of the various mosquito genera and species per house is presented in Table 2.

Table 2: Estimated mean catches per experimental night of the major mosquito genera and species in each of the four study houses caught using HLC during the 8-week study period with 95% CI included. The mean catches were calculated from statistical models.

	An. gambiae s.l.	*An. funestus* s.l.	*An. coustani*	*An. pharoensis*	*Culex* spp.	*Mansonia* spp.
House 1	9.64 (95% CI 8.80-10.55)	5.89 (95% CI 5.24-6.62)	0.81 (95% CI 0.62-1.07)	0.13 (95% CI 0.07-0.26)	8.64 (95% CI 7.95-9.39)	4.49 (95% CI 3.95-5.09)
House 2	6.82 (95% CI 6.13-7.59)	3.82 (95% CI 3.31-4.41)	0.70 (95% CI 0.52-0.95)	0.01 (95% CI 0.001-0.07)	14.44 (95% CI 13.47-15.48)	5.59 (95% CI 4.98-6.29)
House 3	9.47 (95% CI 8.64-10.39)	4.98 (95% CI 4.39-5.65)	2.30 (95% CI 1.92-2.76)	0.34 (95% CI 0.21-0.57)	16.65 (95% CI 15.58-17.79)	3.51 (95% CI 3.02-4.07)
House 4	3.63 (95% CI 3.13-4.20)	4.65 (95% CI 4.08-5.29)	2.37 (95% CI 1.98-2.84)	0.04 (95% CI 0.01-0.12)	15.30 (95% CI 14.30-16.37)	5.20 (95% CI 4.61-5.87)

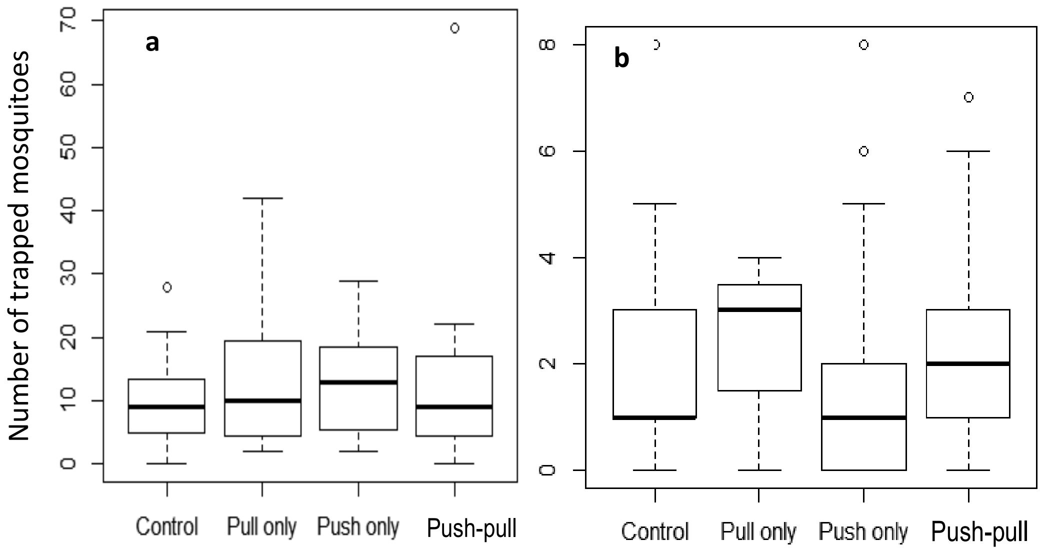

Figure 2. **Outdoor HLC** *Anopheles* mosquito catches in the presence of the four treatments applied. The graphs represent a. *An. gambiae* s.l. and b. *An. funestus* s.l. catches outdoors collected by HLC with the bold line in the box plots showing median catches and the box containing the upper and lower quartiles.

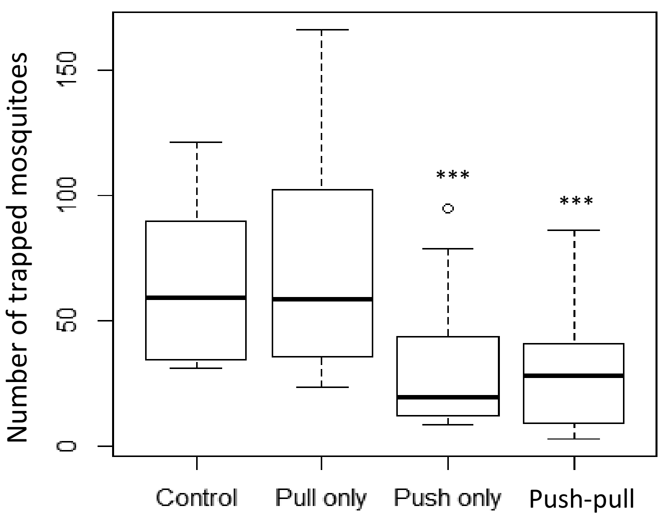

Figure 3. **Outdoor HLC** *Culex* spp. mosquito catches in the presence of the four treatments applied. The box plots represent the median catches (bold line) with the boxes representing both the upper and lower quartiles catches.

Volunteer variations

The four volunteers who participated over the eight weeks attracted variable numbers of mosquitoes per night though none were statistically different. Due to the rotation of treatments in their houses, this variation was adequately catered for.

Outdoor HLC catches

Outdoor HLC catches of malaria vectors, both *An. gambiae* s.l. and *An. funestus* s.l., were not different in the presence of the various treatments compared to the control (Figure 2; Table 3).

In the presence of the push-pull treatment *Culex* spp. mosquito catches were 2-fold lower than in the control ($p<0.001$; Figure 3; Table 3). Catches of *Culex* spp. in the presence of the push only treatment were similar to those obtained in the presence of the push-pull treatment (Table 3).

Table 3: Summary of statistical model outputs for analyses on catches of major malaria vectors, *Culex* spp. and *Mansonia* spp. mosquitoes by **outdoor HLC** in the presence of fabric and Suna trap that were untreated (control), untreated fabric and baited Suna (pull), treated fabric and unbaited Suna (push) and treated fabric and baited Suna (push-pull). All estimated means were calculated from statistical modelling while comparisons of catches in the different traps were made to the catches in the control set up. Figures highlighted in red denote statistical significance.

Female mosquitoes caught outdoors using HLC				
Mosquito species	**Treatment**	**Estimated means**	**Odds Ratio (95% CI)**	**p-value**
An. gambiae s.l.	Control	10.25(95% CI 8.77-11.91)	-	-
	Pull only	14.08(95% CI 12.36-16.04)	1.38 (0.84-2.26)	0.205
	Push only	12.77(95% CI 11.14-14.65)	1.25(0.74-2.09)	0.398
	Push-pull	13.15(95% CI 11.49-15.05)	1.29(0.60-2.75)	0.516
An. funestus s.l.	Control	2.12(95% CI 1.52-2.97)	-	-
	Pull only	2.43(95% CI 1.78-3.33)	1.15(0.64-2.07)	0.649
	Push only	1.87(95% CI 1.31-2.68)	0.88(0.41-1.89)	0.747
	Push-pull	2.37(95% CI 1.73-3.26)	1.12(0.66-1.91)	0.683
Culex spp.	Control	65.01(95% CI 61.18-69.08)	-	-
	Pull only	70.48(95% CI 66.49-74.72)	1.08(0.80-1.48)	0.609
	Push only	31.48(95% CI 28.85-34.35)	0.48(0.32-0.74)	<0.001
	Push-pull	28.80(95% CI 26.29-31.55)	0.44(0.31-0.63)	<0.001
Mansonia spp.	Control	13.56(95% CI 11.89-15-47)	-	-
	Pull only	13.15(95% CI 11.51-15.03)	0.97(0.64-1.48)	0.886
	Push only	9.39(95% CI 8.03-10.99)	0.69(0.47-1.01)	0.057
	Push-pull	10.16(95% CI 8.73-11.82)	0.75(0.52-1.08)	0.126

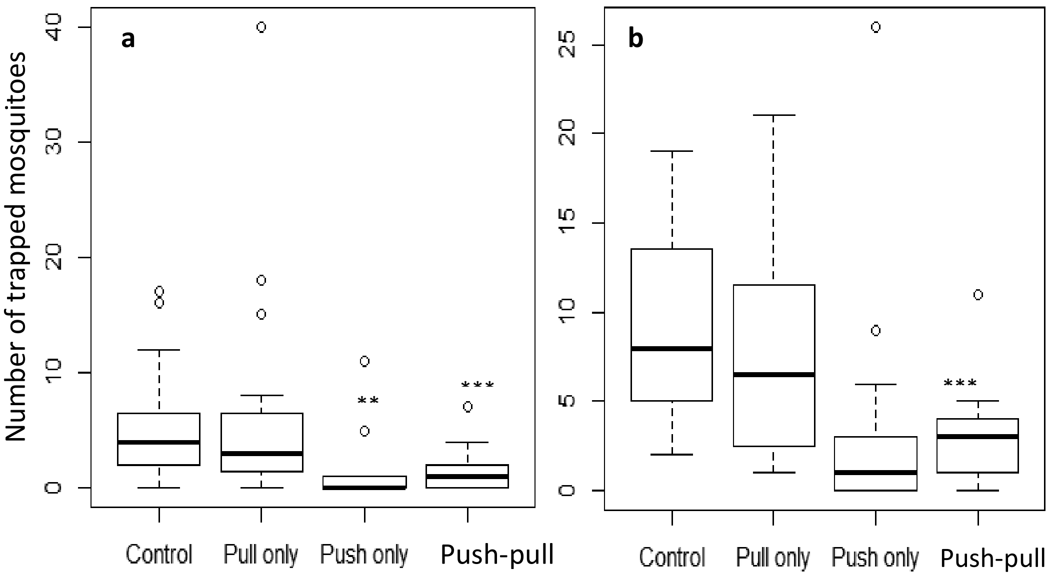

Figure 4. **Indoor HLC** *Anopheles* mosquito catches in the presence of the four treatments applied. The graphs represent a. *An. gambiae* s.l. and b. *An. funestus* s.l. catches indoors collected by HLC with the box plots showing median catches (bold line) as well as upper and lower quartiles.

Indoor HLC catches

In the presence of push-pull and push only treatments, *An. gambiae* s.l. HLC catches indoors were almost 4-fold lower than those in the control treatment (push-pull: p<0.001; Figure 4; Table 4). Catches of *An. funestus* s.l. in the presence of either push-pull or the push only treatments were almost 3-fold lower than those in the control treatment (push-pull: p<0.001; Figure 4; Table 4). Catches of *Culex* spp. in the push-pull treatment were half of those in the control treatment (p=0.136; Table 4).

Table 4: Summary of statistical model outputs for analyses on catches of major malaria vectors and *Culex* spp. mosquitoes by **indoor HLC** in the presence of the four treatments (control, pull, push and push-pull). All estimated means were calculated from statistical modelling while comparisons of catches in the different traps were made to the catches in the control set up. Figures highlighted in red denote statistical significance.

Female mosquitoes caught indoors using HLC				
Mosquito species	**Treatment**	**Estimated means**	**Odds Ratio (95% CI)**	**p-value**
An. gambiae s.l.	Control	5.31(95% CI 4.30-6.56)	-	-
	Pull only	6.71(95% CI 5.56-8.11)	1.26(0.50-3.20)	0.621
	Push only	1.28(95% CI 0.84-1.97)	0.24(0.08-0.72)	0.011
	Push-pull	1.40(95% CI 0.93-2.11)	0.26(0.13-0.52)	<0.001
An. funestus s.l.	Control	9.15(95% CI 7,78-10.76)	-	-
	Pull only	7.30(95% CI 6.09-8.74)	0.80(0.55-1.16)	0.235
	Push only	3.34(95% CI 2.56-4.36)	0.37(0.13-1.02)	0.055
	Push-pull	2.84(95% CI 2.13-3.80)	0.31(0.17-0.56)	<0.001
Culex spp.	Control	3.43(95% CI 2.65-4.44)	-	-
	Pull only	4.33(95% CI 3.43-5.46)	1.26(0.49-3.2)	0.624
	Push only	1.11(95% CI 0.72-1.71)	0.32(0.15-0.69)	0.003
	Push-pull	1.90(95% CI 1.36-2.66)	0.55(0.26-1.20)	0.136

Suna trap catches

During outdoor HLC, Suna trap catches of *An. gambiae* s.l. and *An. funestus* s.l. were lower in the push-pull treatment than in the pull-only treatment, with *An. funestus* s.l. showing a significant difference (p=0.017; Table 5). Similarly, *Culex* spp. mosquito catches were also lower in the push-pull treatment than in the pull only (p<0.001; Table 5).

During indoor HLC, Suna trap catches of *An. gambiae* s.l were comparable in the pull-only and the push-pull treatments (Table 5). Catches of all other mosquito species were slightly lower in the push-pull treatment than in the pull only though were not of statistical significance (Table 5).

Table 5: Summary of statistical model outputs for analyses on catches of major malaria vectors as well as *Culex spp.* and *Mansonia spp.* mosquitoes collected outdoors by Suna traps at the same time as when HLC was being conducted outdoors and indoors. All estimated means were calculated from statistical modelling. Figures highlighted in red denote statistical significance.

	Female mosquitoes caught using Suna trap when HLC is outdoors			
Mosquito species	**Treatment**	**Estimated means**	**Odds Ratio (95% CI)**	**p-value**
An. gambiae s.l.	Pull only	3.87(95% CI 3.02-4.97)	-	-
	Push-pull	3.06(95% CI 2.31-4.05)	0.79(0.48-1.30)	0.351
An. funestus s.l.	Pull only	7.93(95% CI 6.67-9.44)	-	-
	Push-pull	4.75(95% CI 3.79-5.94)	0.60(0.39-0.91)	0.017
Culex spp.	Pull only	6.87(95% CI 5.68- 8.29)	-	-
	Push-pull	2.69(95% CI 2.01-3.62)	0.39(0.26-0.59)	<0.001
Mansonia spp.	Pull only	3.61(95% CI 2.79-4.68)	-	-
	Push-pull	2.17(95% CI 1.56-3.02)	0.60(0.29-1.25)	0.170
	Female mosquitoes caught using Suna trap when HLC is indoors			
An. gambiae s.l.	Pull only	9.11(95% CI 7.75-10.72)	-	-
	Push-pull	9.05(95% CI 7.69-10.65)	0.99(0.63-1.56)	0.976
An. funestus s.l.	Pull only	7.71(95% CI 6.47-9.20)	-	-
	Push-pull	5.41(95% CI 4.39-6.68)	0.70(0.44-1.12)	0.130
Culex spp.	Pull only	6.37(95% CI 5.24-7.75)	-	-
	Push-pull	4.19(95% CI 3.30-5.32)	0.66(0.37-1.17)	0.150
Mansonia spp.	Pull only	3.87(95% CI 3.02-4.97)	-	-
	Push-pull	2.97(95% CI 2.23-3.94)	0.77(0.43-1.35)	0.360

Chapter 5

Discussion

In the present study, the push-pull set up was not associated with a reduction in catches of outdoor-biting malaria vectors. Numbers of other mosquito species, however, specifically those belonging to the genera *Culex* and *Mansonia*, were reduced in the presence of both the push-pull set up and the push only set up.

Indoors, the push-pull set up was associated with reduction in both indoor-biting malaria vectors and other mosquito species, specifically *Culex* mosquitoes. It is possible that the spatial repellent, transfluthrin, in the eave fabrics affected house-entry of mosquitoes leading to lower mosquito catches indoors (Menger *et al.*, 2015; Mmbando *et al.*, 2018; Mmbando *et al.*, 2019; Mwanga *et al.*, 2019). This may be substantiated by the similar levels of indoor protection seen in the present study by both push only and push-pull treatments whose common component is the use of transfluthrin in a passive-release form from the eave fabrics applied on the eave gap. Interestingly though, the indoor reduction in mosquito population was very species-specific in magnitude with *An. gambiae* s.l. showing the largest reduction in terms of magnitude (almost 4-fold) and *An. funestus* s.l. showing the largest reduction in absolute mean numbers. Reduction in indoor numbers of *An. funestus* s.l. shows promise for additional malaria control efforts as this is a major indoor-biting vector (Moiroux *et al.*, 2014; Zhou *et al.*, 2016; Limwagu *et al.*, 2019).

A very similar study had been done in the same area in some of the same houses using a different spatial repellent (delta-undecalactone) in combination with a similar odour-bait as in the present study (Menger *et al.*, 2015). The former study showed a significant reduction in malaria vector indoor catches in the presence of both push only and push-pull control with up to 50% difference in catches with simulations predicting up to 20-fold reduction if applied on large scale (Menger *et al.*, 2015). Unlike the present study, Menger *et al.*(2015) showed significant reduction in indoor malaria vector catches in the presence of pull only set up (Menger *et al.*, 2015).

Outdoor responses of mosquito species to the push-pull set up in the present study showed reduction in numbers of *Culex* spp. and *Mansonia* spp. but not with the malaria vectors. In terms of numbers, *Culex* mosquitoes were the most abundant and in reducing their numbers by more than half could be considered as a benefit against nuisance biting with lower numbers available for the transmission of lymphatic filarial worms and arboviruses (Verhulst *et al.*, 2015; Ogoma *et al.*, 2017; Nchoutpouen *et al.*, 2019).

One possible contributor to the low response among the malaria vectors in the present study could have been that the level of transfluthrin volatilization was insufficient for *Anopheles* mosquitoes, possibly due to lower night temperatures (Ogoma *et al.*, 2017). Transfluthrin requires a minimum ambient temperature to sufficiently volatilize hence the use of electrical volatilisers and burning coils in commercial products where it has been incorporated(Pates *et al.*, 2002; Jeyalakshmi *et al.*, 2014; Ogoma *et al.*, 2014; Bibbs *et* *al.*, 2018; Kwan *et al.*, 2018), as well as better protection seen on days which had higher temperatures (Ogoma *et al.*, 2017).

Very recent studies done in Tanzania that combined transfluthrin-treated eave fabric and BG Sentinel malaria traps showed a higher level of protection against malaria vectors outdoors than in the present study using all the components of push-pull; separately and combined (Mmbando *et al.*, 2017; Mmbando *et al.*, 2019). One main difference in the set ups included the use of 2 traps, 15 m away from the house that resulted in higher protection compared to one and four traps that were tested(Mmbando *et al.*, 2019). Several studies have explored the utilization of different passive emanators for releasing transfluthrin outdoors ranging from chairs, mats and decorations that could be explored instead of the eave fabric (Masalu *et al.*, 2017; Masalu *et al.*, 2018; Masalu *et al.*, 2020).

The contribution of the pull component in reducing catches in the present study was not significant neither for indoor nor outdoor catches. In the cases of *An. gambiae* s.l. and *Culex* mosquitoes, the presence of the pull-only component actually increased the number of mosquitoes caught by the human volunteers indoors. This was an interesting observation considering similar set ups with MB5 supplemented with either CO_2 or 2-butanone had shown significant reductions in house-entry numbers in previous studies (Menger *et al.*, 2014; Menger *et al.*, 2015; Homan *et al.*, 2016; Menger *et al.*, 2016). The explanation for this could be that once mosquitoes were proximal to the house due to the combined attraction of the host cues and artificial lure, the unprotected human provided a more attractive host for the mosquitoes than the trap, directing the mosquitoes to the human (Takken & Knols, 1999; Carde, 2015; Hawkes & Gibson, 2016).

The presence of the human conducting HLC outdoors led to a reduction by one-third of *An. gambiae* s.l. in the Suna trap compared to when the human was indoors. The effect of the repellent in the trap catches was seen in reduced trap catches in almost all species with push-pull in place. But while the trap catches reduced in the presence of the repellent, the human landing catches were similar both in the presence of the repellent alone or a combination of the trap and repellent indicating how much more attractive an unprotected human remains to the mosquito even in the presence of a spatial repellent. The implication of this on the provision of a peri-domestic safe area for unprotected humans would be a requirement for stronger manipulation of host-seeking mosquitoes to sufficiently divert them away from humans and into a trapping device.

In the present study we used four houses and attempted to provide insightful data in natural conditions that can inform larger studies which will in turn generate throughput data to guide the development of an outdoor malaria vector control tool. This study provided substantial information that will guide in the fine-tuning of the push-pull mosquito control tool. One is the consideration of an alternative, more effective spatial repellent that could work effectively against malaria vectors outdoors and an improved pull component that could synergistically work with the repellent and produce a stronger protection than either the repellent or the pull component alone. Additionally, consideration on the production and life-span of the push-pull control products may be made to make them more effective for longer duration and easier to prepare for large scale roll out.

Conclusion:

The transfluthrin/MB5 push-pull system, while not effective at reducing the number of outdoor-biting malaria mosquitoes, caused significant reductions of indoor-biting malaria mosquitoes and therefore provided additional indoor protection. Bites of other mosquito species were reduced both outdoors and indoors. Further research is needed to achieve protection against malaria vectors outdoors with the aim to reduce residual malaria transmission.

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Chapter 6

General Discussion

Chapter 6

General Discussion

This thesis explored the changes in host-seeking behaviour of malaria vectors exposed to spatial repellents and attractants, either separately or in combination, to inform the development of a potential control measure targeting outdoor-biting malaria vectors. Malaria transmission has been shown to be affected by changes in mosquito behaviour, with, in many areas, more mosquitoes caught outdoors than indoors, giving an indication of an increasing role in outdoor-transmission. In western Kenya *Anopheles arabiensis* is the predominant malaria vector outdoors, while *An. funestus* remains the most abundant species indoors (Menger *et al.*, 2015; Degefa *et al.*, 2017; Ogola *et al.*, 2018).

In my study, application of transfluthrin as a spatial repellent on eave fabric significantly reduced the number of mosquito bites both outdoors and indoors. The efficacy of transfluthrin-treated eave fabric in providing significant protection to human hosts outdoors was similar when the eave fabric was presented as a screen or when presented as a strip (partial covering of eave gap). The transfluthrin concentrations in the air declined in a linear fashion from the source of release with higher concentrations detected closer to the ground than higher up. This research has also shown that screening eave gaps with untreated eave fabric confers protection indoors by preventing house entry of malaria vectors. The research in this thesis explored the utilization of a push-pull system; attractant-baited traps in combination with transfluthrin-treated eave strips. However, no additional benefit of the push-pull system over the push only, deployed as transfluthrin-treated eave strips, was seen neither for outdoor nor for indoor protection. The research showed that the odour-baited Suna trap has a better trapping efficiency when supplemented with carbon dioxide than with 2-butanone, a previously reported potential mimic for *An. funestus* s.s. Additionally, my study showed that an attractant-baited Suna trap was not able to divert host-seeking mosquitoes from unprotected humans in a pull-only set up.

The main findings and opportunities for future research arising from this thesis are discussed below.

Malaria control in Africa

Utilization of long-lasting insecticide-treated bed nets (LLINs) and Indoor residual spraying (IRS) in controlling adult malaria vectors has been the core of malaria control and elimination since 2000(WHO, 2019). Since the call to scale up these interventions, over 1 billion LLINs have been distributed in malaria endemic areas leading to a reported 54% coverage in sub-Saharan Africa by 2016(WHO, 2019). This massive effort led to a 62% reduction in *Plasmodium falciparum* malaria mortality in Africa between the years 2000 and 2015 (Bhatt *et al.*, 2015; WHO, 2019). Other intervention indicators such as intermittent preventive treatment in pregnancy and prevalence of severe anaemia also pointed to a decrease in prevalence during this period (Hershey *et al.*, 2017; Kayentao *et al.*, 2018; Wangdi *et al.*, 2018).

Challenges in vector control

After 2015, stagnation occurred in the decrease of mortality rates and in a few worrying cases, increase in infection rates was reported, which adversely affected malaria control efforts(Kiware *et al.*, 2017; WHO, 2019).

Several reasons have been given for the recent lack of progress. Use of LLINs and IRS target indoor-biting mosquitoes and while they reduce malaria incidence, they contribute to growing levels of insecticide resistance and do not offer protection from outdoor transmission (Killeen, 2014; Meyers *et al.*, 2016; Benelli *et al.*, 2017). Physiological changes in mosquito host-seeking behaviour from more predominant late-night and indoor-biting mosquitoes to increasing occurrence of outdoor biting taking place earlier in the evenings or later in the morning (Moiroux *et al.*, 2014; Sougoufara *et al.*, 2014; Mburu *et al.*, 2019) demonstrates the need for supplementary tools (Killeen, 2014; Meyers *et al.*, 2016; Sherrard-Smith *et al.*, 2019) which inspired this thesis.

The overall objective of malaria control efforts is to interrupt parasite transmission, as this is the most effective way to prevent parasites from being transferred to new hosts (Smith *et al.*, 2007). LLINs and IRS have contributed much to reach this goal, but I have outlined that insecticide resistance as well as increased outdoor-biting behaviour are growing obstacles to meet this objective. These developments provide challenges that need to be addressed.

Evaluating putative repellent 'push' and attractive 'pull' components for manipulating the odour-guided orientation of malaria vectors in the peri-domestic space

The natural biting propensity of *An. arabiensis* in western Kenya was explored in Chapter 2 with the findings showing that there was a greater risk of being bitten outdoors than indoors by this particular malaria vector species. The area around the house, referred to as the peri-domestic area, was assumed to be where most of the early evening activities were conducted in rural western Kenya and therefore the area that warranted protection for susceptible humans prior to retreating indoors, where existing tools confer protection (Manin *et al.*, 2016; Finda *et al.*, 2019).

These findings further emphasised the need to develop tools that can be protective against outdoor malaria transmission and that can be supplemented to the indoor tools in use (Benelli*et al.*, 2017).Another major reason for the diminishing gains in mosquito control is the emergence of insecticide-resistant mosquitoes due to the selection pressure applied following constant use of insecticide-based interventions in both agriculture and in public health vector control (Nkya *et al.*, 2014; Benelli *et al.*, 2017; Awolola *et al.*, 2018; Rakotondranaivo *et al.*, 2018; WHO, 2019). Utilization of other insecticide groups has been limited by the high toxicity for mammals and other non-target organisms (Tingle *et al.*, 2003; King *et al.*, 2015; Eldakroory *et al.*, 2017; Han *et al.*, 2018) as well as evidence of pre-existing resistance in malaria vectors against all the major insecticide groups (Hemingway *et al.*, 2000; Cook *et al.*, 2018). Plant-based oils have in the past been used to either repel or kill disease vectors with the added advantage of being environmentally friendly due to being biodegradable, less toxic to humans and non-target organisms and less likely to induce resistance in insects(Diaz, 2016).

I investigated the potential of para-menthane-3,8-diol (PMD) to achieve protection for humans in the peridomestic area as a spatial repellent (Chapter 2). The results showed no apparent repellent effect of PMD on insectary-reared *An. arabiensis*, over a range of concentrations. The most likely reason for this was that PMD in my study did not volatilize to sufficiently high aerial concentrations. In comparison with the Menger *et al.* (2014) study, I relied on passive release of PMD from an eave fabric while the latter utilized multiple mechanical fans to avail PMD spatially(Menger *et al.*, 2014). It appears, therefore, that without this facilitation to release PMD with powered fans, it is more likely that PMD acted as a contact repellent as cited in several publications (Carroll *et al.*, 2006; Diaz, 2016; Colucci *et al.*, 2018; Tangena *et al.*, 2018). PMD is considered to volatilize less than essential oils, making it more likely to offer protection as a topical application to users over a longer period of time (Carroll *et al.*, 2006; Maia *et al.*, 2011). Additionally, as it is derived from plant extracts, PMD is perceived to be safer for use by consumers compared to synthetic repellents while providing protection to a level similar as potent repellents such as DEET (Carroll *et al.*, 2006).

Spatial repellents present more opportunities for application in Africa as they volatilize easily and reduce mosquito-human contact probability while offering protection to both users and non-users at relatively low costs and a low demand on technology (Achee *et al.*, 2012; Norris *et al.*, 2017; Maia *et al.*, 2018).Use of sub-lethal concentrations of insecticides creates opportunities of use as they can evoke a non-toxic but repellent effect on the mosquitoes, which reduces their vectorial capacity while utilizing lower amounts leading to a more cost effective application in the long run(Shaalan *et al.*, 2005; Guedes *et al.*, 2017; Sampaio *et al.*, 2017). Insecticides thus applied may repel mosquitoes or compromise their ability to locate potential human hosts thereby reducing the chances of transmitting diseases without necessarily inducing an immediate toxic or lethal effect(Cohnstaedt *et al.*, 2011; Mbare *et al.*, 2013).On the down-side, it has been shown that sub-lethal concentrations of insecticides could actually increase the fitness of insects such as mosquitoes and subsequently increase their vectorial capacity in transmitting diseases (Margus *et al.*, 2019). Another undesired effect, however, could be the gradual selection of multiple resistance genes in the population as opposed to the selection of one major single resistance gene leading to the undesirable increase of low level resistance mechanisms that may increase in frequency in the population(Guedes *et al.*, 2017). Thirdly, it is reported that sub-lethal concentrations could increase the risk of major resistance developing in the population eventually as opposed to if there would have been no selection pressure(Gressel, 2011).

Transfluthrin, a fast-acting pyrethroid, has been used in commercial products such as mosquito coils and electric vaporizers and has been shown to volatilize easily and act as both repellent and a killing agent (Nazimek *et al.*, 2011; Jeyalakshmi *et al.*, 2014; Ogoma *et al.*, 2014b). Due to unavailability of power sources to run electric vaporisers in rural areas and in order to reduce risks involved in the inhalation of toxic smoke when burning coils, the passive release of transfluthrin impregnated in locally available hessian fabric has been explored for both indoor and outdoor protection with marked success (Masalu *et al.*, 2018; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019a; Masalu *et al.*, 2020).

In Chapter 2,I examined the utilization of a sub-lethal concentration of transfluthrin, applied on an eave fabric for passive release to achieve protection against *An. arabiensis* in the peri-domestic area. Frequency of mosquito bites outdoors in the peri-domestic area was significantly reduced in the presence of transfluthrin-treated eave fabric.

Due to previous studies that pointed to the risk of spatial repellents diverting mosquitoes to unprotected persons when coverage in a community was incomplete(Moore *et al.*, 2007; Maia *et al.*, 2016), the inclusion of a trap baited with an attractive lure in combination with the spatial repellent was explored in a “push-pull” set up. This was heavily inspired by the push-pull strategy developed for application in agriculture where the use of a non-preferred plant species intercropped with a target crop plant (e.g. maize) diverted stem borer moths away (push) and to an alternative ‘trap’ plant (pull) (Cook *et al.*, 2007; Khan *et al.*, 2016).

Host-seeking malaria mosquitoes have been known to locate their potential blood hosts based on the detection of carbon dioxide and a range of other physical and chemical cues released naturally by the host that the mosquito has evolved to detect when in need of a blood meal (Takken *et al.*, 1997; Takken *et al.*, 1999; Ray, 2015).Following identification of key chemicals involved in host-seeking behaviour, the

Mbita blend 5 (MB5) was developed and used in traps together with carbon dioxide for effective surveillance and control of malaria vectors (Mukabana *et al.*, 2012; Mweresa *et al.*, 2015).

In Chapter 2 it was shown that the MB5 lure in combination with carbon dioxide did not confer protection to susceptible humans outdoors. The attractiveness of the human in the presence of the synthetic lure was significantly higher and thus the odour-baited trap did not reduce the human landing catches in the system. In the absence of a human, the odour-baited trap, however, captured at least one-third of all released mosquitoes, which was consistent with previous studies (Hiscox *et al.*, 2014; Mburu *et al.*, 2017).

Supplementation of odour-baited traps with carbon dioxide has presented challenges in the field as the acquisition and proper storage of the gas in any form is logistically demanding. This necessitated the exploration of carbon dioxide mimics (Turner *et al.*, 2011; Mburu *et al.*, 2017). One mimic, 2-butanone, showed promising results in field studies (Turner *et al.*, 2011; Hiscox *et al.*, 2016; Homan *et al.*, 2016; Mburu *et al.*, 2017). Replacement of carbon dioxide with 2-butanone showed significantly lower *An. arabiensis* catches (Chapter 2),which was not comparable to catches obtained with carbon dioxide alone or when carbon dioxide was combined with the MB5 lure and as such2-butanone was not considered an adequate mimic for carbon dioxide for the capture of *An. arabiensis*. Previous studies have implied that use of 2-butanone as a mimic of carbon dioxide with MB5 lure in Suna traps may be more attractive to *An. funestus* and was able to effectively reduce its population size following mass trapping(Homan *et al.*, 2016; Mburu *et al.*, 2017).

A combination of repellents and attractants has been proposed to potentially synergize the protective effect conferred by each alone and in turn to provide increased protection against both indoor and outdoor-biting mosquitoes (Takken, 2010; Menger *et al.*, 2014; Mmbando *et al.*, 2019).

The combination of transfluthrin-treated eave fabric and MB5-baited Suna trap led to a reduction in the proportion of outdoor-biting *An. arabiensis* but was not better than the repellent alone (Chapter 2). Therefore, I concluded that the combination of transfluthrin and an odour-baited trap did not result in additional protection against outdoor-biting *An. arabiensis*. In line with my results, recent studies done in Tanzania showed only a modest additional protection conferred by the push-pull set up against outdoor-biting mosquitoes compared to the push only set up (Mmbando *et al.*, 2019).The inclusion of an odour-baited trap may not have provided additional personal protection due to its proximity to both the repellent and the human. In Chapter 3, the influence of the spatial repellent on trapping effectiveness of the odour-baited trap was demonstrated when recapture rates dropped from 30% to under 5%. Based on this and observations made in other studies (Menger *et al.*, 2015; Mmbando *et al.*, 2019), the odour-baited traps should be positioned further away from the house and monitored more on a community-level – users and non-users alike, as opposed to direct personal protection(Mmbando *et al.*, 2019). This was further shown from mathematical simulations developed to determine the value of push-pull with impact at community level being the main focus(Menger *et al.*, 2015).

Detection of mosquito control chemicals in the air provides additional data on the actual concentrations presented to mosquitoes as well as offer information on the levels of exposure as part of public health advise on safety (Martin *et al.*, 2013; Ogoma *et al.*, 2014a; Kwan *et al.*, 2018). In Chapter 2, quantification of chemicals in air samples obtained in the push-pull system showed an approximately linear reduction in transfluthrin concentration from the point of release outwards and upwards with lowest concentrations being detected at the furthermost point of sampling and at higher points of sampling. MbitaBlend-5 (MB5) chemicals were only sporadically detected. Availability of the chemicals in the air as confirmed in Chapter 2 further confirms the possibility of presenting the push-pull control to mosquitoes outdoors and influencing their approach to the human host. Future prospects are improvement of the push-pull mosquito control tool by ensuring that effective concentrations are reached separately and when combined preferably with synergistic effects to reduce host biting frequency. Utilization of active dispensers, especially for the spatial repellent, would further increase the release rate and increase aerial concentration though this would inadvertently increase the costs and technical requirements making this an inhibiting factor for large-scale roll out for malaria control.

Sight versus smell: Dynamics of malaria mosquito attraction response in the presence of spatial repellents

Host-seeking mosquitoes utilize both physical and olfactory cues detected by different sensory pathways in order to successfully locate a potential host for a blood meal (Cardé *et al.*, 2010; Takken, 2011; Hawkes *et al.*, 2017). Repellents have been shown to work by interfering with host-seeking mosquitoes' perception of human odours through either olfactory masking or undefined "irritation" of mosquitoes such that the frequency of contact with a potential human host is either reduced or is avoided altogether, leading to a reduced probability of a successful blood-meal(Bohbot *et al.*, 2010; Murphy *et al.*, 2013; Ray, 2015; Tzotzos *et al.*, 2018). Further, it has been shown that in many cases, compounds that at relatively low concentrations act as attractants, can function as repellents at higher concentrations indicating a concentration-dependent switch of olfactory perception resulting in attraction or repellency(Semmelhack *et al.*, 2009; Ray, 2015).Application of both repellents and attractants in a push-pull set up towards synergizing the protection conferred by each individual component against outdoor-

biting mosquitoes (Chapter 2) could inadvertently cause diminished perception of the attractive lure by the olfactory neurons(Bohbot *et al.*, 2010; Tsitoura *et al.*, 2015; Tzotzos *et al.*, 2018).
Utilization of a different attractive cue such as artificial light in the form of the CDC UV light trap was proposed in my study to be used as the pull component in the push-pull system due to its trapping efficiency seen in various studies(Sexton *et al.*, 1986; Rubio-Palis, 1996; Mwanga *et al.*, 2019b).As nocturnal mosquitoes can identify their hosts without artificial light, the selection of light as an attractive cue was an arbitrary one and future exploration of an alternative cue or a combination of attractive cues that could work best in the presence of a spatial repellent is warranted. It is also possible that the odour bait can be made more attractive by varying the composition or concentration of the chemical components.

In Chapter 3 it is demonstrated that mosquito catches in the odour-baited trap were reduced significantly in the presence of the transfluthrin repellent as compared with the absence of this repellent, while mosquito catches in the CDC UV light trap remained relatively similar both in the absence and presence of this repellent.MB5-baited traps may be unsuitable for use in close vicinity to a spatial repellent in a push-pull device as the spatial repellent may interfere with the mosquito's perception of synthetic lures. Positioning the traps further from the repellent source may improve the trapping efficiency in an area where mosquitoes can detect and respond to the synthetic lures dispensed by the trap. UV light traps might be more efficient and potentially easier to manage with constant trapping potential even in close proximity of a spatial repellent; though in my study the proportion of trapped mosquitoes was too low to consider this a potential pull component. Since both trap types were not very efficient in trapping mosquitoes in the presence of a spatial repellent, especially in the absence of a human, there is an urgent need to develop more attractive traps that can be combined with spatial repellents in a push-pull setup to improve efficacy.
Host-seeking mosquitoes constantly fly upwind towards a source of carbon dioxide and only perceive additional attractive odours and other cues once in proximity of the host (Spitzen *et al.*, 2013; Cardé, 2015). Entering a repellent plume may momentarily interrupt the tracking of odour cues but by flying randomly in and out of this plume, a mosquito could once again determine the source of the attractive odour (Cardé *et al.*, 2010; Achee *et al.*, 2012; Hawkes *et al.*, 2017). By positioning the trap further from the repellent plume, like 7.5 m or 10 m, it may improve the location of the source of the synthetic lure by the host-seeking mosquito and improve the overall impact of the push-pull system. This hypothesis may be corroborated by the fact that momentary exposure to the repellent plume did not have any effect on the proportion of mosquitoes caught in either the odour-baited trap or CDC UV light trap.

Chapter 6

Mosquitoes have species-specific responses to chemical compounds, demonstrated in various studies (Afify *et al.*, 2020; Martin *et al.*, 2020; Nararak *et al.*, 2020). Findings in Chapter 3 support the differential response of sibling species to repellents and attractants and provide an indication of how highly specific chemosensory perception is among closely related mosquito species. This creates both obstacles and opportunities in vector control towards ensuring that tools generated are not only effective against one target species but could potentially work against vectors having different physiological traits to achieve a broad applicability.

Less is more: repellent-treated eave strip as a substitute for eave screen for indoor and outdoor protection of malaria mosquitoes

Open eaves have been shown to contribute significantly to the house-entry of malaria mosquitoes with subsequent increase in malaria transmission and by simply sealing eaves, the risk is eliminated (Njie *et al.*, 2009; Ogoma *et al.*, 2010; Mburu *et al.*, 2018). Improvement on house structure to ensure that entry ways used by mosquitoes to access houses have been either completely sealed or reduced substantially have been shown to reduce populations of malaria vectors indoors (Kaindoa *et al.*, 2018; Mburu *et al.*, 2018; Ngadjeu *et al.*, 2020). Further evidence of this was seen in Chapter 4 when untreated screens on eaves significantly reduced indoor biting populations of malaria vectors with no anticipated effect on outdoor biting mosquitoes. This was expected as the untreated screens provided a physical barrier that prevented mosquitoes from accessing the house confirming the importance of sealed eaves in malaria control (Menger *et al.*, 2016; Tusting *et al.*, 2017; Getawen *et al.*, 2018).

Passive release of spatial repellents enables utilization in endemic areas with minimal need for re-treatment or regular compliance by users and no requirement for provision of electrical power that may not be available or affordable in some rural settings (Ogoma *et al.*, 2017; Moshi *et al.*, 2018). Impregnation of hessian cloth with transfluthrin and utilization as an eave fabric applies low technology using locally available natural fibre and provides effective protection for up to six months; both indoors and outdoors (Ogoma *et al.*, 2012; Govella *et al.*, 2015; Masalu *et al.*, 2017).

In Chapter 4 it was shown that passive release of transfluthrin from the eave fabric provided protection from indoor-biting mosquitoes in semi-field conditions. There was an indication that temperature played a role in protection conferred outdoors, with more protection being seen when ambient temperatures were higher and a reduction of protection being seen at lower temperatures. The effect that temperature had on protection conferred by transfluthrin was cited in a study by Ogoma *et al.*(2017) who stated that when preceding daily temperatures fell below 23 °C, protection conferred by transfluthrin was greatly reduced(Ogoma *et al.*, 2017).

Presentation of the treated eave fabric as a strip (partial covering) as opposed to a screen (full coverage) significantly reduced both the number of mosquito landings indoors and outdoors. The interesting observation was that despite the remaining eave gaps, transfluthrin released from the eave strip was sufficient to disrupt host-vector interaction both indoors and outdoors, to a similar extent as treated eave screens. The advantage of using eave strips as opposed to eave screens would potentially be lower costs if scaled up since the amount of fabric used is less than half that used in the screening and since perfect fitting for each house is not a requirement, it results in ease of application without the need for customization per house and reduced skilled application(Lindsay *et al.*, 2003; Njie *et al.*, 2009; Ogoma *et al.*, 2010).

Pre-evaluation of push-pull mosquito control under natural conditions in western Kenya

Due to evidence obtained in the semi-field conditions as described in Chapter 2, a field experiment was conducted in western Kenya to test the performance of the transfluthrin/MB5 push pull system under natural conditions and to determine how the separate components of the tool perform in comparison to when combined as a way to gather research-based evidence for their recommendation and to direct future research and large-scale trials(Takken, 2010; Menger *et al.*, 2015; Mmbando *et al.*, 2019).As semi-field experiments were limited to only two sibling mosquito species (*An. arabiensis* and *An. gambiae s.s.*), the field set up was specifically designed to investigate how mosquitoes belonging to diverse mosquito genera and species would respond to the push-pull set up. Ultimately, products developed for vector control need to be applied in natural environments and so their performance in the field informs this application.

In Chapter 5 it was shown that significantly fewer malaria mosquitoes were caught indoors in the presence of both the push-pull and push only set ups indicating protection achieved by their application. Indoor transmission of malaria still accounts for the majority of malaria transmission (Bayoh *et al.*, 2014) and so additional protection from indoor-biting malaria vectors can contribute to the reduction in malaria prevalence. However, the increasing role of outdoor malaria transmission remains unaddressed by the transfluthrin/MB5 combination of the push-pull set up and would therefore warrant additional research to remedy this.

A previous study done in the same area showed a reduction in number of indoor-caught mosquitoes with all the applied treatments (repellent only – delta undecalactone, attractant only – MB5-baited MMX trap and the two treatments combined) (Menger *et al.*, 2015). Our study showed a similar trend with the significant reduction in *Anopheles* species indoors in the presence of the repellent only and when combined with the attractant, but there was no effect with the attractant only(Jawara *et al.*, 2009; Menger *et al.*, 2015).

The odour-baited trap in our study had previously been used for mass-trapping mosquitoes in western Kenya that led to significant reductions in *An. funestus* populations and consequently, reduction in malaria cases (Homan *et al.*, 2016). The possibility of a species-specific response to the odour-baits is plausible as in our study a slight reduction in *An. funestus* populations was seen indoors in the presence of the MB5-baited Suna trap but not in any of the other mosquito species. If the study had continued for a longer period and with more households as was the case in the Homan *et al.* (2016) study, it may be possible that this would have led to significant reductions in *An. funestus* numbers (Homan *et al.*, 2016). As currently the most abundant malaria vector is *An. funestus*, reductions in its numbers would positively contribute to efforts in malaria control, especially in western Kenya where indoor transmission still accounts for most of malaria transmission (Degefa *et al.*, 2017; Ogola *et al.*, 2018).

Another study done in Central America evaluating combining indoor applied transfluthrin with an outdoor odour-baited CDC light trap demonstrated a species-specific reduction in indoor entry of *Anopheles* species, especially due to the repellent only (Wagman *et al.*, 2015). While the results of this study closely mirror ours, the diminished role that push-pull played in indoor entry of mosquitoes was more evident in the Wagman *et al.* (2015) study than it was in ours.

Positioning of the push-pull components appears to contribute positively to the outcome based on comparisons made with a recent field study done in Tanzania that showed a reduction in outdoor-biting by *An. arabiensis* when the repellent was actively emitted at least 5 m from the house and the pull component positioned at least 10 m from the repellent(Mmbando *et al.*, 2017).Preliminary results from Chapter 3 in this thesis indicate a possible interference in performance by odour-baited traps in the presence of a repellent, especially when in very close proximity of the repellent plume detected in Chapter 2. By positioning the trap further away from this plume, this may enhance its overall contribution to the push-pull strategy.

Another major difference between the Tanzania study and our study was the active release of transfluthrin using powered fans, which could have facilitated increased aerial distribution of transfluthrin that may have increased protection conferred(Mmbando *et al.*, 2017). While the use of active emanators might be a limiting factor due to unavailability of electric power in many malaria endemic areas in sub-Saharan Africa as well as high cost of the equipment especially for large scale application, it may be interesting to consider additional sources of cost-effective passively-emitted repellent for example from baskets and decorations placed inside or near the homes in addition to the eave fabric as an alternative to further improve the outcome(Masalu *et al.*, 2018).

Non-malaria vectors in our study were more responsive to the transfluthrin/MB5 set up outdoors as their proportional landing catches were significantly reduced both in the push-pull set up and in the presence of the repellent only. A number of studies have suggested that both *Culex* and *Mansonia* species are not very host-specific in their quest for blood meals given their non-specific host selection (Muturi *et al.*, 2008; Garcia-Rejon *et al.*, 2010; Telang *et al.*, 2019). These species could possibly be reduced by this tool to improve control of transmission of arboviruses and endophagic filariasis and to reduce nuisance-biting by mosquitoes. Although these mosquito species also readily enter houses and would probably equally have been affected by the transfluthrin, in the present study their densities were too low for a statistical evaluation.

Future perspectives

The findings in this thesis provide several opportunities to further develop and improve the control of malaria mosquitoes and present possible advancements in addressing hurdles in malaria control.

Manipulating odour-orientation of malaria vectors in the peri-domestic space using push-pull strategy

While the importance of outdoor transmission of malaria is not questioned(Degefa *et al.*, 2017; Sougoufara *et al.*, 2020), development of vector control tools targeting outdoor-biting mosquito populations has not yet resulted in inclusion in malaria control programmes(WHO, 2019).The use of transfluthrin as a spatial repellent passively emanating from eave fabric reduced the number of mosquito bites outdoors in semi-field conditions (Chapters 2 and 4) but not in natural conditions (Chapter 5). Likely reasons for this were the requirement of higher temperatures required to volatilize transfluthrin that are usually lacking during the hours when the vectors are active in various endemic areas(Ogoma *et al.*, 2017).

One remedy would be to use a spatial repellent that volatilizes more efficiently at lower temperatures occurring in the evenings and night in many endemic areas, and is preferably a non-pyrethroid compound to avoid the contribution to selection of multiple resistant genes when used over time(Guedes *et al.*, 2017).One possible spatial repellent would be delta-undecalactone that had shown promise when used as an eave fabric both in semi-field (Menger *et al.*, 2014; Menger *et al.*, 2016) and in field assays(Menger *et al.*, 2015). Other volatile organic compounds that present a similar repellent effect on mosquitoes as well as possible blends of non-pyrethroid chemicals could also be considered for optimized testing and application that could also affect pyrethroid resistant mosquitoes. In addition to their application on eave fabric, the integration of additional points of emissions for the spatial repellents such as sisal baskets and functional hangings positioned further from the house could be explored to increase the space in which the repellent concentration is above the effective threshold in the peri-domestic area.

Another improvement on the present study would be the microencapsulation of the spatial repellent as opposed to impregnation (Miro Specos *et al.*, 2016). Transfluthrin in this thesis was impregnated into hessian strips (Ogoma *et al.*, 2012; Ogoma *et al.*, 2017)which, while it was not tested for association with experimental outcomes, could present an issue in the large scale roll out of this tool. By inclusion of the repellent as microcapsules in the eave fabric, the preparation of the repellent prior to use of fabric in the field could be commercialized for scaling up with uniformity of repellent load per fabric guaranteed(Menger *et al.*, 2015; Miro Specos *et al.*, 2016; Misni *et al.*, 2017). An unfortunate downside to microencapsulation is that, being a high-tech application, it may potentially increase both the short and long-term costs in large scale application unless a more affordable option is generated that would be just as effective and safe for use(Misni *et al.*, 2017).

Attractive pull component

The pull component of the set ups tested in this study did not confer protection of human hosts indoors or outdoors, in field or semi-field conditions. An attempt to replace the odour-baited trap with a light trap as described in Chapter 4 did not improve the trapping efficiency in the presence of the spatial repellent, transfluthrin.

As a way forward, an improved trap, possibly an odour-baited one with additional host cues such as warmth and humidity (Hawkes *et al.*, 2017; Cribellier *et al.*, 2020)may be considered while also exploring its placement further away from the repellent plume – possibly between 7.5 m and 20 m in the direction of breeding habitats for more efficient trapping of mosquitoes either separately or in combination with the spatial repellent (Okumu *et al.*, 2010). Additionally, the placement of the pull component could be explored to provide community protection as opposed to household protection (Homan *et al.*, 2016) to further improve protection and potentially lower costs.

Protective eave fabric

In this thesis, transfluthrin was used on eave fabric and presented either as an eave strip (partial covering) or eave screen (full covering). Results obtained in this study (Chapter 4) provide evidence of similar effectiveness when using eave strips to replace eave screens with comparable protection obtained both indoors and outdoors(Govella *et al.*, 2015; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019a).

As a way forward, eave fabric could be applied as repellent-treated strips and offer the same level of protection as treated eave screens. Ultimately, improvement of housing would be ideal (Atieli *et al.*, 2009; Tusting *et al.*, 2017; Kaindoa *et al.*, 2018; Mburu *et al.*, 2018) though in low income areas where this may not be an immediate solution, the eave strips will provide adequate and easy to apply protection to both users and non-users (Mwanga *et al.*, 2019a).

Conclusions

In conclusion, this thesis has shown that i) repellent-treated eave strips provide protection from malaria vectors; both indoors and outdoors, with possibilities of significant cost-effectiveness in scale-up exercises compared to repellent-treated eave screens, ii) push-pull control provides an opportunity for outdoor control of nuisance-biting non-malaria mosquitoes and possibly reduce transmission of arboviruses and lymphatic filariasis, iii) the current push-pull strategy is not yet effective against malaria vectors outdoors under natural conditions and iv) a more effective attract and kill tool against host-seeking malaria vectors than the ones tested in my study would possibly improve the protection conferred by the push-pull mosquito control tool.

Future research should focus on a) identification and utilization of potent volatile compounds with high vapour pressure whose application is not restricted to climatic conditions specific for certain geographic regions, b) diversifying from odour-baited traps as attractants to more robust multi-cue traps to further optimize protection of susceptible human hosts outdoors and c) optimizing combination of the spatial repellent with a very attractive mosquito trap to achieve a maximum synergistic performance by investigating the placement geometry and d) possibilities for community effect push-pull set ups could be explored where design is done such that some households employ the repellent only while others strategically utilize attractant-baited traps for possible improvement of outdoor protection.

Summary

The urgency to develop new tools to supplement the currently existing ones; namely LLINs and IRS, has been fuelled by recent stagnation in reducing prevalence and incidence of malaria especially in sub-Saharan Africa. This stagnation has been attributed to increasing insecticide resistance in major malaria vectors and changes in mosquito populations; from predominately endophilic and anthropophilic to exophilic and opportunistic feeders. The rising importance of outdoor-biting malaria vectors in maintaining residual malaria transmission has warranted the development of control tools that target them while supplementing the existing tools. Due to the mainly indoor protection conferred by LLINs and IRS, development of new control tactics is required to target mosquito populations that bite and rest predominately outdoors. Tools previously developed to potentially target malaria mosquitoes when used singly were experimentally combined towards synergizing their protective efficacy outdoors. Spatial repellents have in recent years received renewed interest due to their ability to prevent host-vector contact and can be used to control outdoor-biting mosquitoes and in many cases, act on mosquitoes shown to be insecticide resistant. When used in combination with odour-baited traps, spatial repellents present opportunities for mosquito control by applying a proposed ‘push-pull’ strategy in controlling outdoor-biting mosquitoes that could potentially contribute to the control of residual transmission.

In order to understand the dynamics that a push-pull mosquito control strategy would involve, research questions were studied; 1) Does the inclusion of an optimally-performing spatial repellent in an eave fabric reduce outdoor-biting rates of malaria vectors? 2) Does the addition of an optimized odour-baited trap to the spatial repellent improve on any protection conferred? 3) Are the airborne concentrations of the chemical components of the spatial repellent and odour trap quantifiable towards determining effective range of possible protection?

In **Chapter 2**, I outlined systematic investigations of different concentrations of PMD and transfluthrin as potential spatial repellents applied on eave fabric and their protective efficacy on unprotected persons in the peri-domestic space outdoors quantified in semi field systems in western Kenya. Two concentrations of PMD tested did not offer any protection while two concentrations of transfluthrin were both protective, with the higher concentration offering more protection than the lower one, confirming the spatial repellency effect of transfluthrin on *Anopheles arabiensis*. Supplementation of transfluthrin with MB5-baited Suna trap did not have any effect on the protective efficacy seen. The MB5-baited Suna trap on its own did not divert mosquitoes from an unprotected human confirming that an unprotected human remains more attractive to host-seeking malaria vectors than to synthetic lures. Utilization of 2-butanone as a supplement to MB5-baited Suna was not comparable to addition of carbon dioxide indicating that 2-

butanone is not an adequate carbon dioxide mimic in attracting *An. arabiensis*. Quantification of the airborne concentrations of the push-pull chemical components showed that transfluthrin was detectable within a 5 m radius from the eave fabrics applied on experimental houses, with higher concentrations being detected nearest the house and closer to the ground with reducing concentrations further away and higher up in the treated space.

In **Chapter 3**, I explored the influence that transfluthrin as a spatial repellent has on the trapping efficiency of a MB5-baited Suna trap compared to a CDC UV light trap as a basis of considering alternative pull components in the push-pull system. In the absence of transfluthrin, the odour-baited Suna trap caught more *An. arabiensis* and *An. gambiae s.s.* than the CDC UV light trap indicative of the strong and specific attractive lure offered by the odour traps that is absent in the light trap. In the presence of transfluthrin, a significant reduction in the catches was seen in the odour trap which was indicative of the effect that transfluthrin had on the mosquito perception of synthetic lures from the odour trap. Trapping efficiency of the light trap remained fairly constant even in the presence of transfluthrin as mosquito perception of light remained unaffected in the presence of spatial repellents. The efficiency of the light trap was generally quite low and as such, use of a light trap as a potential pull component could not be considered.

In **Chapter 4**, I investigated the plausibility of presenting transfluthrin on an eave fabric that partially covered the eave gap as a strip instead of the traditional screen that requires complete coverage of the eave gap. Transfluthrin-treated eave strips provided similar protection to unprotected persons, both indoors and outdoors, as transfluthrin-treated eave screens providing a more cost-effective solution especially when scaled-up due to the utilization of less fabric and increasing the ease of application. Additionally, my investigations were able to reiterate the importance of eave screening as application of an untreated eave screen significantly reduced indoor-biting mosquitoes.

In **Chapter 5**, I carried out field evaluations of the push-pull strategy in a village in western Kenya to establish its performance in natural conditions. The application of the transfluthrin-treated eave fabric on its own significantly reduced the number of *An. gambiae* s.l. and *An. funestus* s.l. caught indoors. The inclusion of an odour-baited Suna trap did not have an additional effect on the protection conferred by transfluthrin indoors. Non-malaria vectors were significantly reduced outdoors, both in the presence of the repellent alone or repellent and odour-baited trap. None of the primary malaria vectors had a difference in their catches in the presence of any of the treatments applied.

The final chapter of this thesis provided a general discussion of the observations seen in the various chapters as well as conclusions obtained.

Summary

In summary, push-pull strategy presents a tool that can control outdoor-biting mosquitoes and may need to be optimized to effect a protective action against malaria vectors in various geographical and climatic conditions. Also, presentation of spatial repellents in an eave fabric that does not fully cover the eave gap is just as protective as a repellent-treated eave screen and could present opportunity for cost-effective control of malaria in areas where improvement of housing is not immediately possible. Generation of a more effective attract and kill tool that can supplement transfluthrin would improve the overall performance of the push-pull strategy and in the long run, identification of an even better spatial repellent that is effective against both malaria and non-malaria vectors in various geographical conditions would be ideal in ensuring effective disease control.

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List of Abbreviations

CDC UV	Centre of Disease Control light trap with ultra violet light
DDT	Dichlorodiphenyltrichloroethane
DEET	N,N-Diethyl-meta-toluamide
GC-FID	Gas Chromatography coupled with Flame Ionisation detector
GEE	Generalized estimating equations
GLMER	Generalized linear mixed effects models
HLC	Human landing catches
IRS	Indoor residual spraying
LED	Light-emitting diode
LLINs	Long-lasting insecticide bed nets
MB5	Mbita blend 5
OR	Odds ratio
PMD	Para menthane-3,8-diol
WHO	World Health Organization

List of publications

Njoroge M.M., Fillinger U., Saddler A., Moore S., Takken W., van Loon J.J.A., Hiscox A. Evaluating putative repellent 'push' and attractive 'pull' components for manipulating the odour orientation of host-seeking malaria vectors in the peri-domestic space. Parasites &Vectors. 2021 Jan 11; 14(1):42. doi: 10.1186/s13071-020-04556-7. PMID: 33430963; PMCID: PMC7802213.

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Margaret Mendi Njoroge

Curriculum Vitae

Margaret Mendi Njoroge was born in Nairobi, Kenya on 9^{th} June 1979. After undertaking both her primary and secondary education, she joined Maseno University for a Bachelors of Science in Biomedical sciences and technology and majored in Medical biotechnology. Immediately after this, Margaret received training at the Kemri-Wellcome Trust Research group on *Plasmodium falciparum* point mutations that lead to drug resistance. This was followed by an intensive 2-year work contract at the Med Biotech Laboratories in Kampala, Uganda where drug resistance mutations in eastern Uganda were studied. She undertook her masters' studies in Molecular Biology at the VrijeUniversiteit Brussel (VUB) in Belgium on a VLIR-UOS scholarship. Her masters' thesis was on Vaccination of promiscuous peptides and dynamics of myeloid cells in experimental models of murine schistomiasis. After her masters' studies, she worked as a project administrator at the African Academy of Sciences. Together with her former Ugandan supervisor, she secured a Grand Challenges Exploration grant on maternal immunization for the protection of neonates and infants from malaria at the Institute of Primate Research (IPR). Since 2012, she has been working at the International Centre of Insect Physiology and Ecology (*icipe*) as a research assistant on various human health projects including treatment of cattle with insecticide for control of mosquito vectors and most recently the East African collaboration on mosquito push-pull, which looked at combined use of spatial repellents and attractants to control outdoor-biting malaria vectors. This was also the project that sponsored her admission into the PhD graduate programme at the Wageningen University and Research (WUR). Her PhD thesis has been on understanding the host-seeking behaviour of *Anopheles* mosquitoes in response to olfactory and visual cues. Margaret is currently involved in a One-Health project on the use of biorational solutions for controlling human and animal disease vectors in Kwale and Busia counties in Kenya.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Background literature on chapters of PhD thesis: host-seeking behaviour of *Anopheles* mosquitoes in response to olfactory and visual cues

Post-graduate courses (7 ECTS)

- R Statistics for arthropod research; ICIPE (2017)
- Vector ecology and disease training; ICIPE (2018)
- International Chemical Ecology (ICE) course; ICIPE/Max Planck/Penn State/SLU (2019)

Deficiency, refresh, brush-up courses (12 ECTS)

- Ecological aspects of biointeractions; WUR (2017)
- Ecological methods; WUR (2017)

Laboratory training and working visits (1.9 ECTS)

- Design of semi-field systems for testing push-pull mosquito control experiments; Ifakara Health Institute, Bagamoyo, Tanzania (2017)

Competence strengthening / skills courses (3.7 ECTS)

- Research proposal writing course; International Foundation for Science/ICIPE (2015)

Scientific integrity / ethics in science activity (1.8 ECTS)

- Online course on research ethics; Global Health Network (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (1 ECTS)

- PE&RC First years weekend (2017)
- WGS/PE&RC Last stretch of the PhD programme (2019)

National scientific meetings / local seminars / discussion groups (9.8 ECTS)

- Mbita science club meetings; ICIPE (2017-2019)

- One health entomology group meetings during periods spent; WUR (2017, 2019)
- Scientific meetings: malaria, human health and all themes; ICIPE (2017-2019)

International symposia, workshops and conferences (6.3 ECTS)

- 5th Pan African Mosquito Control Association (PAMCA); Victoria Falls, Zimbabwe (2018)
- 12th Neglected Tropical Diseases (NTD) conference; Nairobi, Kenya (2018)
- 11th European Congress of Tropical and International Medicine (ECTMIH); Liverpool, United Kingdom (2019)

Societally relevant exposure (0.3 ECTS)

- Wellcome-Trust malaria program for primary students documentary interview (2019)

Lecturing / supervision of practicals / tutorials (3.6 ECTS)

- World malaria day-community training (2017, 2019)
- Malaria introductory course for primary students (2019)

MSc thesis supervision (6 ECTS)

- Use of transfluthrin shields in the control of outdoor malaria transmission
- Inferiority trials on LUMIN8 light trap against standard CDC light traps on major malaria vectors in western Kenya
- Attractiveness of MB5 odour-bait in comparison to humans to the major malaria vectors in western Kenya
- Quantification of protection conferred against indoor-biting malaria vectors using controlled release design (insecticide) repellent blocks

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