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ABSTRACT Synthetic versions of human derived kairomones can be used as baits when trapping host seeking mosquitoes. The effectiveness of these lures depends not only on their attractiveness to the mosquitoes but also on the medium from which they are dispensed. We report on the development and evaluation of nylon strips as a method of dispensing odorants attractive to the malaria vector, *Anopheles gambiae* s.s. (Giles). When a synthetic blend of attractants was dispensed using this method, significantly more mosquitoes were trapped than when two previous methods, open glass vials or low density polyethylene sachets were used. We conclude that the nylon strips are suitable for dispensing odorants in mosquito trapping operations and can be adopted for use in rural and remote areas. The nylon material required is cheap and widely available and the strips can be prepared without specialized equipment or electricity.

KEY WORDS synthetic lures, trapping methods, *Anopheles gambiae*, malaria, odor dispensing methods

Behaviorally active odor compounds are important in various applications including the control of mosquitoes that transmit malaria. These compounds influence insect behavioral responses toward hosts, mates, or other resources such as oviposition sites and sugar sources (Zwiebel and Takken 2004). For example, malaria vectors use a selection of host derived cues to locate suitable humans from which they take blood meals. Recent research has focused on the development of new public health products, which consist of synthetic attractants as the active ingredients. Already several chemicals from human body odor, sweat, and breath have been identified and proven to attract a variety of mosquito species (Cork and Park 1996, Healy and Copland 2000, Bernier et al. 2002, Healy et al. 2002). These compounds can be synthesized artificially and used as attractants of disease transmitting arthropods such as the African malaria vector, *Anopheles gambiae* (Giles) (Cork and Park 1996). Other applications might include baiting of insecticides to improve their effectiveness; as proxy hosts to replace human volunteer subjects in testing of topical repellents; or as barriers that disrupt normal host seeking behavior to prevent contact between the vector and susceptible humans.

The activity of insect attractants depends on the amount and the concentration of volatiles released in the environment as well as the medium from which they are released (Torr et al. 1997). Improved results can therefore be achieved from systems that provide sustained release of optimal odor concentrations for extended periods of time. These attributes can be guaranteed by selecting appropriate material media from which to dispense the odorants. Essentially, the medium should be odorant-permeable and should confer minimal change on the chemical characteristics of the active ingredient. Other important attributes of an appropriate delivery medium include the ease of preparation and availability especially where large quantities of material are required for field operations.

We developed and evaluated a simple method that is cheap and easy to prepare and used it to successfully dispense synthetic odorants for trapping malaria mosquitoes. The method involves using nylon fabric that has been impregnated by soaking in solutions of mosquito attractants, as a delivery medium to dispense the attractants. The technique was inspired by the method of collecting human foot odors using worn nylon socks, a technique previously used to trap *An. gambiae* s.s. mosquitoes (Njiru et al. 2006, Schmied et al. 2008). This paper presents results of semifield experiments in which we compared the nylon strips with two previously used odor dispensing methods, open glass vials and low density polyethylene (LDPE) sachets, and with worn nylon socks. The open glass vials technique

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was earlier used in studying olfactory responses of *An. gambiae* to different synthetic odor blends (Okumu 2008) while LDPE sachets have previously been evaluated and used as methods for dispensing tsetse fly attractants (Torr et al. 1997).

Materials and Methods

Origin of the Nylon Strips Concept. We initially used worn nylon socks as a method of collecting human foot odors. This method was based on findings by DeJong and Knols in the 1990s (de Jong and Knols 1995, Knols 1996) that foot odors are highly attractive to *An. gambiae.* These authors also found that washing feet reduced attractiveness to the mosquitoes. Foot odors collected in worn nylon socks were first tested in the semifield system (Njiru et al. 2006), who concluded that the worn socks were more attractive to host seeking mosquitoes than ammonia or carbon dioxide. Human skins uniquely secrete triglycerides that are broken down into fatty acids by various skin microflora (Cork and Park 1996, Bernier et al. 2002, Natsch et al. 2006) and some of these fatty acids are known to attract mosquitoes (Cork and Park 1996, Bernier et al. 2000, Qui 2005, Bernier et al. 2007). An important characteristic of the compounds is that they are highly volatile and can penetrate and become trapped in any odor-permeable medium; such as socks worn on human feet or fabric wrapped around ankles.

Foot odors collected in worn nylon socks were initially used as a standard against which the attractiveness of different synthetic odorants were assessed (Okumu 2008). In those experiments, the odorants were dispensed using open glass vials fitted onto the Mosquito Magnet X (MMX; American Biophysics Corporation, North Kingstown, RI) trap, a counter flow geometry trap (American Biophysics Corporation). One obvious inconsistency with this method was that the worn socks had greater surface area of exposure than the open glass vials. Another problem was that to test an odor-blend consisting of several different constituents, one would need a lot more vials than could possibly be inserted into the MMX trap. This means that even though the MMX is an efficient trap for host seeking mosquitoes (Cooperband and Carde 2006, Njiru et al. 2006, Qiu et al. 2007, Schmied et al. 2008), baiting it with synthetic odor blends especially those consisting of many distinct attractants can be difficult and may impede adequate airflow through the attractant plume tube. As described later under the subsection 'Tests to compare the nylon strips with other odor dispensing methods,' to evaluate the behavioral responses of mosquitoes to our test blend, which consisted of multiple constituents, we initially had to use customized odor dispensing methods so as to allow the required number of vials to be used with the MMX trap, but that unfortunately had the potential of distorting airflow within the attractant plume tube of the trap. Similarly, low density polyethylene bags, which have previously been used to dispense tsetse fly attractants (Torr et al. 1997), proved immensely cumbersome for purposes of dispensing synthetic odor blends from the MMX trap. The number of sachets that are required to dispense the blend meant that the remaining open space within the attractant plume tube of the MMX trap was also too limited to allow efficient air flow.

Therefore, the challenge was to standardize the method of releasing the odorants so that it would be as similar as possible to the worn socks while at the same time ensuring that it could be incorporated into the MMX functional mechanism. Our solution was to infuse a piece of nylon with the synthetic odorants and then compare its attractiveness to that of a worn nylon sock with equal size.

To test a blend consisting of several compounds, a piece of nylon would be cut into as many equal-sized strips as the constituents of the test blend. Each strip would then be soaked in the different constituents. Using this procedure, we achieved the same surface area as the worn nylon socks, as long as we used a piece of nylon that was originally the same size as the worn sock. This method also meant that the strips could then be easily bundled up and suspended into the MMX trap plume tube in the same way as the worn socks (Figs. 1 and 2). Therefore, this paper reports on the use of nylon strips infused with attractants not only as a new method of dispensing blends of mosquito attractants but also as a new method of baiting MMX traps with synthetic attractants.

Mosquitoes. The experiments were conducted using laboratory reared *An. gambiae* s.s. that had originally been obtained from Njagi village in Kilombero district, southern Tanzania. The larvae were fed on Tetramin fish food and at maintained at temperature of 27 ± 1°C. Adult mosquitoes were kept inside mosquito cages measuring 30 × 30 × 30 cm in a separate room, where temperatures were maintained at 27 ± 1°C and relative humidity at 70–90%. The adults were fed on 10% glucose solution delivered through Watman filter paper. The insectary was set to 12D:12L.

The Semifield System. Experiments were conducted within a semifield enclosure (screen house), at the Ifakara Health Institute (IHI). The screen house had three equal-area compartments each measuring 200 m^2. Only one of the components was used for this research. The walls were made of mosquito-proof gauze allowing for outside air to flow freely inside it. The roofing was translucent but rainproof while the floor was earthen and void of any vegetation (Ferguson et al. 2008). In the hours between 1900 h and 0700 h when all the experiments were conducted, wind was mainly still inside the screen house while temperature and relative humidity ranged between 16 and 24°C, and between 57 and 84%, respectively. These were generally comparable to night time environmental conditions outside the screen house. Finally, all lights were switched off during the times when experiments were running.

Mosquito Collections. The MMX, a counter flow geometry trap made by the American Biophysics Corporation was used to comparatively evaluate the mosquito responses elicited by the odor compounds (Kline 1999, 2002). The MMX trap (Fig. 2) consists of

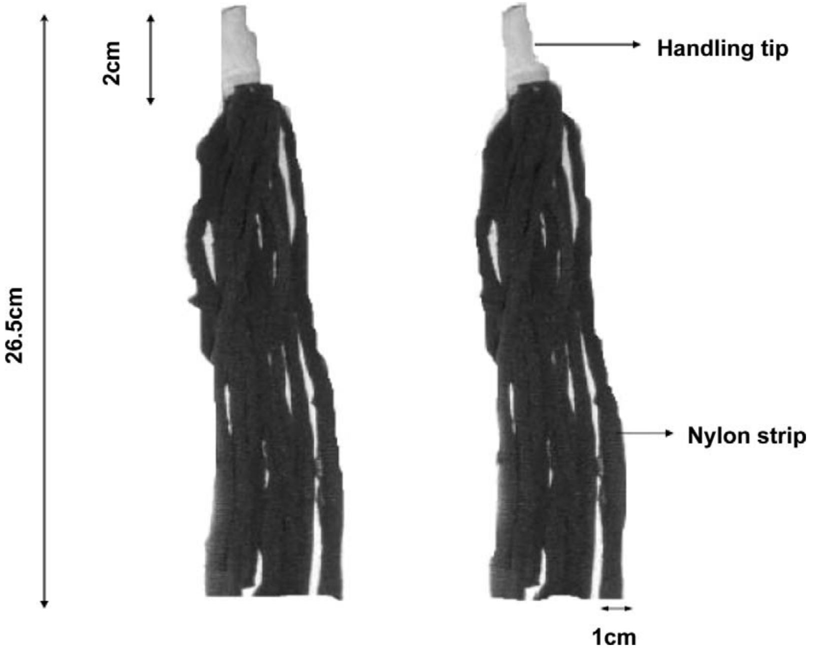

Fig. 1. Batches of nylon strips prepared for dispensing mosquito attractants. Each strip is soaked in solution of a different constituent of the test blend. The strips are bundled in such away as to enable their insertion into the attractant plume tube of the MMX trap. One batch of strips is used to bait the trap by suspending it using a thin nylon string inside the attractant plume tube of the trap, so that it is directly under the attractant plume fan as shown in Fig. 2.

an oval shaped plastic casing (the collection container) enclosing extended inner tubing where the bait is inserted (the attractant plume tube). It has two fans blowing air in opposite directions. The smaller fan (the attractant plume fan) located directly on top of the attractant plume tube blows air out. Simulta-

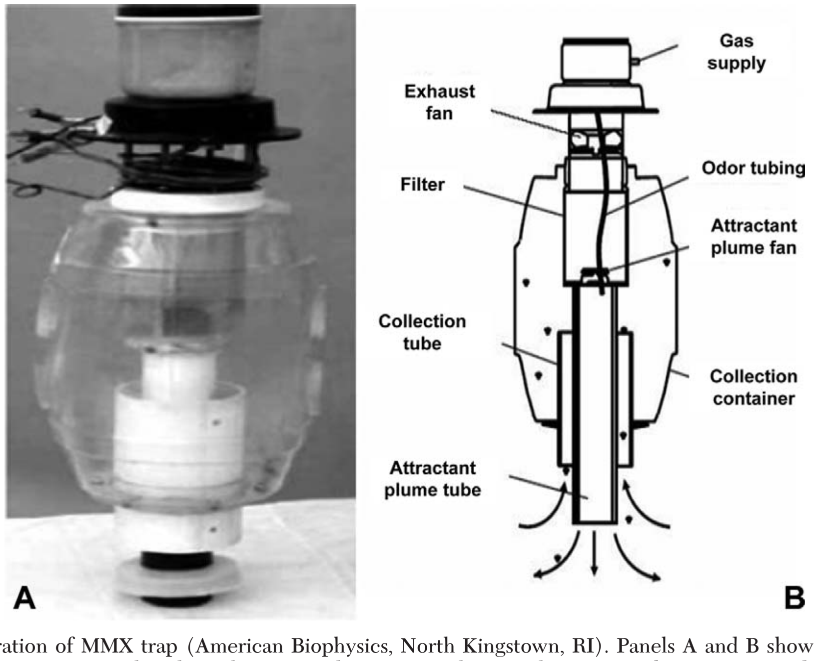

Fig. 2. Illustration of MMX trap (American Biophysics, North Kingstown, RI). Panels A and B show the picture and drawing of the trap, respectively. The nylon strips, the LDPE sachets or the worn socks were inserted into the trap by suspending them using small nylon strips inside the attractant plume tube so that air expelled by the attractant plume fan could blow uniformly over them.

neously, the larger fan (the exhaust fan) that is located near the top of the trap sucks air upwards through the trap, thereby creating a counter current suction mechanism. Attracted mosquitoes trace the path of the expelled air current, which carries the volatiles from the bait. When they reach near the lower end of the trap, they are sucked into the collection container by the more powerful current of the exhaust fan. At the end of the experiment, the collection tube is closed using a plastic seal after which the trap is disconnected from the 12 volt battery that powers it.

Preparation of the Nylon Strips. We used nylon fabric made of 15 denier microfibers. The textile composition was 90% polyamide and 10% spandex (Bata Shoes, Kenya). We first cut the nylon into narrow strips each measuring 26.5 cm in length and 1.0 cm in width (Fig. 1). Each strip was then soaked into separate solutions of the attractants. Though the strips were soaked until they were saturated with the odorant solutions, no attempts were made to estimate the total amount of compound infused inside them. A blend of synthetic odor compounds that was recently developed at the Ifakara Health Institute was used as the test blend for this study (F. O. et al., unpublished). In experimental hut studies conducted in a malaria endemic village in southern Tanzania, this blend was demonstrated to effectively mimic real human odors and to be consistently more attractive to host seeking mosquitoes than humans are over long range distances (F. O. et al., unpublished, patent pending). The blend consisted of optimum concentrations of synthetic versions of human derived mosquito attractants: carbon dioxide, ammonia, L-lactic acid and seven other aliphatic carboxylic acids namely: propionic acid, butanoic acid, pentanoic acid, 3-methyl butanoic acid, heptanoic acid, octanoic acid and tetradecanoic acid (patent pending). To formulate the required concentrations, these constituents were all diluted in distilled water except octanoic acid and tetradecanoic acid that were diluted in ethanol. Carbon dioxide, the only gaseous constituent of the blend was delivered directly to the MMX trap using rubber tubing at a rate of 500 ml/min, measured using calibrated gas flow meters (Glass Precision Engineering Ltd., United Kingdom). Therefore, to deliver the liquid constituents of the test blend, a batch of 10 nylon strips was required each time.

Ammonia was available as 25% aqueous solution while L-lactic acid was available as 85% pure solution also prepared in water. The other carboxylic acids were available as absolute formulations with purity levels ranging between 97 and 99%. However, the CO_2 gas was available only as industrial grade and therefore its exact purity could not be ascertained. Even though high volatility is an important physical characteristic of mosquito attractants (Takken and Knols 1999, van der Goes van Naters and Carlson 2006), no attempts were made to measure the volatility of the odorant compounds making up our test blend. All the compounds were purchased from Sigma Aldrich (Germany), except CO_2 that was supplied by Tanzania Oxygen Company Ltd.

To ensure consistency of data, nylon strips sufficient to supply one complete experiment were made up at the same time. The strips were left inside the glass bottles containing the odor solutions and kept at 4°C for as long as the experiments lasted (1 to 2 wk). To make up the test blend on the day of the experiment, one strip was picked from each of the solutions of the constituent odorants. Excess solution was squeezed off using flat tip tweezers after which the strips were suspended on metal racks for between 4 and 6 h at room temperatures. This ensured that the strips were not dripping with fluid but were instead semidried at the start of the experiments. The tweezers were cleaned with acetone and dried with cotton wool to ensure no cross contamination. The strips were then batched together and kept in labeled polythene bags awaiting insertion into the MMX traps. A 2 cm handling tip was made using a nonvolatile adhesive tape on one end of each batch of strips (Fig. 1). To prevent contamination of the traps or odor dispensing media with human odors, clean latex gloves were worn during the preparations as well as during the baiting of the MMX traps. Similarly, the traps were thoroughly cleaned using 10% acetone solution at the end of each test. To bait the MMX traps, the batch of nylon strips was fitted inside the attractant plume tube of the trap, so that it was hanging directly in the path of the air expelled by the attractant plume fan (Fig. 2).

Assay Procedures. In all tests, a binary set up was used with two MMX traps, fitted with any two different odor dispensing methods, were placed inside the screen house compartment at two opposite corners so that the traps were 20 m apart. The traps were suspended on tripod stands so that their lowest part was at a height of 15 cm from the ground. Each night, 200 female *An. gambiae* s.s. mosquitoes (not previously blood fed) were released from a central point, 10 m equidistant from the two traps. No attempts were made to determine whether the selected female mosquitoes had been mated or not. In addition, because only females were used for the experiments, there was no possibility of mating once the mosquitoes were selected. The selected mosquitoes were gently aspirated into smaller cages, using plastic tubes fitted with rubber tubing, ensuring that the mosquitoes did not die during the process. Between 6 and 8 h before each test, the mosquitoes were starved by withdrawing their sugar meals and water. To release the mosquitoes, the netting of the small cage was opened up and the cage was gently shaken to let out the mosquitoes. Two tests were performed per night (between 1900 to 0100 h and again between 0110 to 0710 h), each time introducing a fresh bait. No attempt was made to recapture mosquitoes that had not been caught during the first phase of the night before releasing additional mosquitoes for the second phase, as this would be impractical considering the large size of this screen house chamber. Because of this, and also because there were generally fewer mosquitoes responding during the first phase than during the second phase, the mosquito catches in either of the two traps have been represented as proportions of all responding

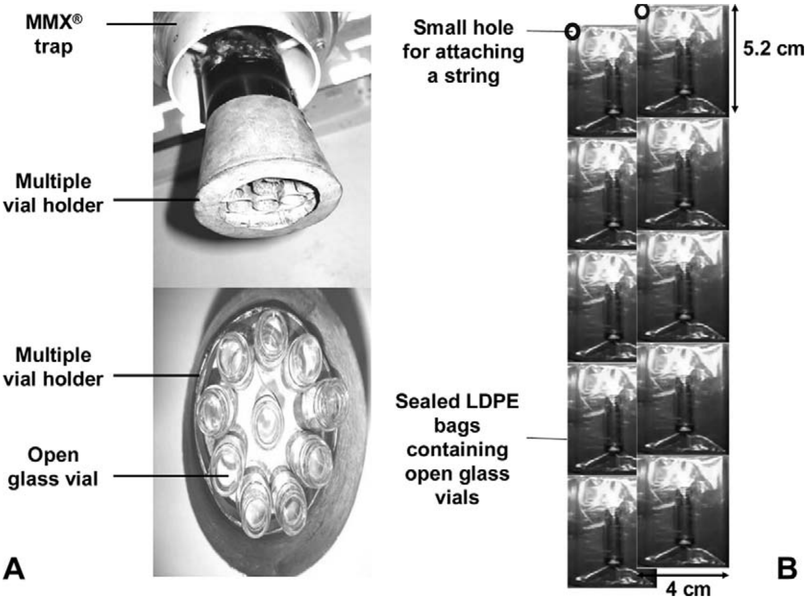

Fig. 3. Open glass vials inside a multiple vial holder (panel A) and LDPE sachets (panel B) used for dispensing constituents of the test blend. The multiple vial holder carries 10 open glass vials and was attached to the lower end of the attractant plume tube. However, the LDPE sachets in two stacks of 5 units each were suspended using nylon threads inside the attractant plume tube.

mosquitoes rather than as absolute numbers of mosquitoes. Thus, only the responding mosquitoes (but not all mosquitoes released) were considered in comparing any two odor dispensing methods. This is explained in further detail under the sub section titled 'statistical analysis.'

Tests to Compare the Nylon Strips With Other Odor Dispensing Methods. To assess the efficiency of the nylon strips, we compared the attractiveness of the test blend when delivered through nylon strips against the attractiveness of the same blend when delivered through either open glass vials (Wheaton, Millville, NJ) or low density polyethylene (LDPE) sachets (Rapoport, Northbrook, IL). Aliquots (1 ml) of the individual constituents of the test blend were added into 4 ml glass vials. Each open glass vial had radius of 0.5 cm, meaning that the exposed surface area of the solution inside it was 0.785 cm^2. The vials were arranged inside a customized vial holder and used to bait the MMX trap (Fig. 3a). This vial holder, fabricated from aluminum sheets, had originally been used with MMX traps to enable testing of synthetic odor blends consisting of multiple constituents (Okumu 2008), but was obviously disadvantageous as it could distort the of odor plumes coming out of the traps. The second MMX trap was baited with a bundle of 10 nylon strips; each strip soaked in a different constituent of the test blend constituent (Fig. 1). Carbon dioxide gas was added to both traps at a constant rate of 500 ml/min. Eight replicates of the experiment were conducted each time swapping the positions of the two dispensing methods. The number of mosquitoes collected in the two traps was used as an estimate of the attractiveness of the bait and therefore as an indicator of the suitability of the dispensing method.

Similarly to compare the nylon strips with the LDPE sachets, one of the MMX traps was baited with a bundle of nylon strips soaked in the constituents of the test blend while the second MMX trap was baited with LDPE sachets inside which there were open glass vials each containing 1 ml of separate solutions of the odor compounds (Fig. 3b). The packed sachets were then suspended inside the attractant plume tube of the MMX trap using a nylon thread so that the expelled air could blow uniformly over them. The LDPE sachets were 0.02 mm in thickness and had a total surface area of 41.6 cm^2 each. As in the previous experiment, carbon dioxide was added to both traps at a constant rate of 500 ml/min. Eight replicates were conducted, each time swapping the positions of the two dispensing methods. To ensure that the odorants did not contaminate the traps, latex gloves were worn when baiting the traps. In addition, the MMX traps were marked so that one particular trap was always used with one particular dispensing method. Each morning the traps were thoroughly cleaned using 10% acetone solution prepared in water.

Tests to Compare Nylon Strips With Worn Nylon Socks. Natural human foot odors were collected by wearing a nylon sock for a period of 10 h before the experiment. Socks were worn by one of the investigators (L.B.). The worn sock was used to bait one of the MMX traps by suspending it in the attractant plume tube as previously reported (Njiru et al. 2006, Schmied et al. 2008). The second MMX trap was baited with a bundle of nylon strips containing the test blend as in the previous experiments. Fourteen replicates

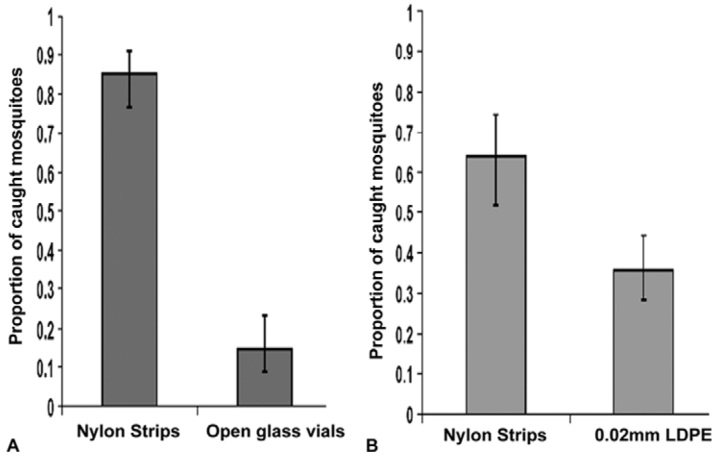

Fig. 4. The proportion of caught mosquitoes that are found in MMX traps baited using nylon strips when compared in binary assays against traps baited using open glass vials (panel A) or traps baited using 0.02 mm low density polyethylene sachets (panel B). The Y-error bars represent the 95% confidence intervals.

were conducted each time swapping the positions of the two baits.

Statistical Analyses. We analyzed the data using SPSS version 15 (SPSS Inc., Chicago, IL). The number of mosquitoes caught in the traps was used as an indicator of the relative efficiency of the dispensing method. The preference by mosquitoes to fly to either the treatment trap (the trap containing the synthetic blend delivered through nylon strips) or the control trap (the trap containing only the synthetic blend delivered through either LDPE, open glass vials or worn socks) was coded as 1 and 0, respectively. The preferences were then weighted by the number of mosquitoes caught per trap per replicate. The proportion of mosquitoes caught in the treatment trap (P_t) was modeled for each comparison using binary logistic regression. P_t was estimated as a function of the categorical variables, trap location (x_1) and phase of the night (x_2). We used a stepwise backward-conditional method to assess the significance of the independent variables before inclusion in the regression models. The intercept obtained was exponentiated to determine the odds of mosquitoes being caught in the treatment trap. We then used the odds to estimate the probability that the mosquitoes would be trapped preferentially when the test blend is dispensed using nylon strips as opposed to any other test blend, that is, estimated probability = Odds/(1 + Odds). Because mosquitoes released during the first phase of the night but not trapped at that time could possibly respond during the second phase of the night, this method of using binary proportions of mosquitoes responding in each phase as opposed to all mosquitoes released, was preferred because it allowed for data from each phase to be statistically analyzed as distinct replicates.

Results

When the test blend was dispensed using nylon strips, significantly more mosquitoes were trapped than when either open glass vials ($P < 0.001$) or low density polyethylene sachets ($P = 0.019$) were used. The average number of mosquitoes trapped per test using the nylons strips and the open glass vials were 17 and 45, respectively, meaning that of the 200 mosquitoes released, an estimated 30.6% were recaptured. However, the average number of mosquitoes trapped using nylon strips and the 0.02 mm LDPEs were 14 and 39, respectively, which translate 26.1% recapture rate of all mosquitoes released.

Logistic regression analysis, correcting for the effects of location of traps and phase of the night, showed that the attractiveness of the blend (and 95% confidence interval) was 5.83 (3.29–10.33) times higher when dispensed using the nylon strips than when it was dispensed using open glass vials (Fig. 4a). However, the blend was 1.78 (1.09–2.91) times more attractive when delivered using the nylon strips than when it was delivered using 0.02 mm-thickness LDPE sachets (Fig. 4b). In another experiment, traps baited using the test blend dispensed from nylon strips caught 46.54% (38.37–54.89%) of all responding mosquitoes compared with 53.46% (45.11–61.63%) caught when the traps were baited using worn nylon socks plus CO_2, a nonsignificant difference ($P = 0.408$).

Discussion

There is growing interest on human derived odorants that can be harnessed to obtain high impact products for controlling important disease vectors. Therefore, it is important that appropriate lure dispensing technologies are also researched as this will accelerate the implementation of odor based technologies and associated operations. Through this study, we have shown that nylon strips are effective for dispensing synthetic chemicals that attract host seeking mosquitoes. The material is cheap, readily available, and easy to prepare and store and is therefore suitable for use even in remote and rural field settings. We found that by dispensing the odorants using the nylon strips instead of either open glass vials or low density polyethylene sachets, the attractiveness of a synthetic odor blend to the malaria vector *An. gambiae* s.s. could be improved several fold.

One explanation for the improved attractiveness of the odor blends when dispensed using the nylon strips is the differences in exposure area. The strips obviously exposed the odorants on a larger surface area than either the open glass vials or the LDPE sachets. In addition, increased number of either the vials of the LDPE bags meant that the area available for air flow as well as the speed of the air within the attractant plume tube was reduced. However, using the nylon strips ensured that the unoccupied open area within the attractant plume tube was still enough to ensure adequate airflow. Our results therefore highlight a limitation of using glass vials and LDPE bags for dispensing blends with several constituents, especially when the MMX is the trap of choice. However, we recognize that the two methods may remain effective when only a small number of different odorants are to be dispensed.

The use of LDPE sachets to dispense insect attractants was previously investigated by Torr et al., 1997, who reported that the rates of odorant flow depended directly on temperature and the surface area exposed but also inversely on the thickness of the LDPE bags (Torr et al. 1997). Our results indicate that significantly more mosquitoes were trapped when nylon strips were used than when a 0.02 mm thickness LDPE sachet was used ($P = 0.019$). Though we did not test different thicknesses of LDPE sachets, we infer from the study by Torr et al. 1997, that the odor release rates would be lower if the synthetic lure were delivered using thicker sachets. In fact, at 0.02 mm, the sachets we used were thinner than all those tested by Torr et al. (1997).

Open glass vials have also been successfully used to deliver synthetic odorants for attracting *An. gambiae s.s.* (Njiru et al. 2006, Okumu 2008). It is similarly expected that better release rates are achievable when the surface area exposed to the air current is large. In Okumu (2008), it was reported that the same test blend attracted 16.7% (12.20–22.55%) of all responding mosquitoes when it was dispensed using open glass vials and tested in a competitive binary assay against worn socks and CO_2 gas. In this study, we report a greater relative efficiency of 46.54% (38.37–54.89%) when the same blend is dispensed using nylon strips. Though we may attribute these improvements to greater surface area of the nylon strips, it is not possible to accurately predict how changing these release rates actually affect the mosquito responses. This is because some behaviorally active odorants may exhibit both attractive and repellent properties (Okumu 2008). Nonetheless, increased release rates may generally be associated with increased mosquito catches as long as the attractive range of concentrations is not exceeded.

The technique of impregnating fabric with behaviorally active chemicals is widely used in organic chemical industries but has also been successfully applied in public health to deliver insecticides against biting arthropods. Familiar examples include in insecticide treated nets (Yates et al. 2005), insecticide treated curtains (Crook and Babtista 1995), and insecticide impregnated clothing (Kimani et al. 2006) that are common interventions against malaria vectors. Some scientists have also experimented with the impregnation of insect repellents into nets (Kawada et al. 2005, N′Guessan et al. 2006). A common challenge to scientists working with odor baited technologies, is how to ensure that the active ingredient last as long as is operationally desirable. In our experiments, tests were conducted for only 6 h and therefore we had no immediate requirement for a permanent impregnation method. We achieved impregnation by simply soaking the strips in the odorant solutions until the fibers were saturated. We recognize however that it may be necessary in other operations for suitable fixing agents such as resins to be added to ensure the active ingredient lasts longer. The resins should preferably be inert so as not to elicit any behavioral responses from the target vectors.

However, for use in sampling the strips may be prepared as pretreated fabric in vacuum sealed sachets to preserve them before use in the field. The nylon strips are easier to prepare and to use in field settings, they cannot break, or spill or puncture, and are seven times and five times cheaper than vials and LDPE sachets, respectively. Moreover, even though the new method was developed for use with a specific trap, the MMX, the strips are flexible and may be bundled in different conformations and used with several other traps.

One potential disadvantage of the nylon strips is that they tend to dry up quickly and therefore there may be no more active ingredient dissipating from the material after some hours. Control operations would require the incorporation of better impregnation techniques. However, the nylon strips were most appropriate for experiments where synthetic odor blends need to be released for only a limited period of time such as for mosquito sampling.

This new odor dispensing method has already been adopted for use in a number of field experiments involving synthetic odor blends (F. O. et al., unpublished data). In one study, the method was successfully used to dispense odorants in experimental huts so as to compare the attractiveness of a synthetic odor blend to attractiveness of humans. That experimental hut study demonstrated that when synthetic odor blends similar to the one used here was dispensed using nylon strips, significantly more mosquitoes were lured into experimental huts than when human volunteers slept in the huts. In addition, in the same study, the nylon strips were used to test whether the same synthetic blend could be used to reduce exposure of humans to disease carrying mosquitoes at house hold level (F. O. et al., unpublished data). In a third example, we used the nylon strips as a dispensing medium in experiments where the synthetic odor blend was evaluated as a replacement for human volunteers in efficacy trials of topical repellents (Okumu et al. 2009).

This research was focused primarily on comparative evaluation of nylon strips against the two previously used methods, open glass vials and LDPEs, with regard to their suitability for dispensing odorants from the MMX trap. The nylon strips are suggested as a suitable alternative for used especially in field based studies in which synthetic odor blends with multiple constituents are required to attract host seeking mosquitoes. Because our experiments were conducted only with the MMX trap, these results may not be directly applied to other traps except where the functional mechanisms are similar. The open glass vials and the LDPE bags may therefore remain appropriate for use with several other traps especially those into which several individual odorants may be inserted without the limitation of space for airflow.

One limitation of this research was the low recapture rates observed in the semifield system when using laboratory reared *An. gambiae* s.s. mosquitoes. Even though this study enabled determination of which of any two odor dispensing methods was more appropriate to use with the MMX trap, additional data regarding response rates of wild mosquitoes in field settings are necessary to corroborate these results. In our own field trials conducted in a malaria endemic village in southern Tanzania, considerably high numbers of mosquitoes were caught using this method. An average of 333 female *An. gambiae* s.l. mosquitoes per night were collected inside huts in which the same test blend used here was dispensed using the nylon strips (F. O. et al., unpublished data). The same field study also demonstrated that this test blend, dispensed using nylon strips, was significantly more attractive to host seeking mosquitoes (patent pending). Finally, like in most studies where synthetic odorants have been tested as attractants for host seeking mosquitoes, carbon dioxide was an important component of our test blend. It was useful to activate the mosquitoes and as a synergist to improve attractiveness of the other compounds (Gillies 1980, Gibson et al. 1997, Dekker et al. 2005, Schmied et al. 2008). In this study, it was also the only constituent of the blend that could not be dispensed through either nylon strips or vials or the polyethylene sachets, and was therefore delivered directly through rubber tubing.

We conclude that nylon strips are a suitable method for dispensing odorants and can be easily incorporated into mosquito traps to disperse attractants. Industrially produced odorant-dispensers may be expensive, may require specialized equipment or are designed to dispense only individual compounds as opposed to blends of compounds. The nylon material used for making the strips is cheap and readily available. The strips can also be readily prepared without specialized equipment or electricity and can be used to deliver blends of multiple compounds. Therefore, the method is appropriate for field operations including in rural and remote areas. However, more permanent impregnation techniques will be necessary if the method is to be applied for delivering odorants over extended periods of time.

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