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Development of Fungal Applications on Netting Substrates for Malaria Vector Control

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ABSTRACT Mosquito resistance to chemical insecticides is considered a serious threat for the sustainable use of contemporary malaria vector control methods. Fungal entomopathogens show potential as alternative biological control agents against (insecticide-resistant) anophelines. This study was designed to test whether the fungus, *Beauveria bassiana*, could be delivered to mosquitoes on netting materials that might be used in house screens, such as eave curtains. Tests were conducted to determine effects of formulation, application method, netting material, and nature of mosquito contact. *Beauveria* had a twice as high impact on *Anopheles gambiae s.s.* longevity when suspended in Shellsol solvent compared with Ondina oil (HR = 2.12, 95% confidence interval = 1.83–2.60, $P < 0.001$), and was significantly more infective when applied through spraying than dipping. Polyester and cotton bednets were the most effective substrates for mosquito infections, with highest spore viability on cotton nets. Whereas fungal impact was highest in mosquitoes that had passed through large-meshed impregnated nets, overall efficacy was equal between small- and large-meshed nets, with ≤30-min spore contact killing >90% of mosquitoes within 10 d. Results indicate that the use of fungal spores dissolved in Shellsol and sprayed on small-meshed cotton eave curtain nets would be the most promising option for field implementation. Biological control with fungus-impregnated eave curtains could provide a means to target host-seeking mosquitoes upon house entry, and has potential for use in integrated vector management strategies, in combination with chemical vector control measures, to supplement malaria control in areas with high levels of insecticide resistance.

KEY WORDS vector control, *Beauveria bassiana*, *Anopheles gambiae*, house screening, entomopathogenic fungi

Contemporary malaria vector control methods focus on providing protection from infectious mosquito bites using fast-killing chemicals on bednets long-lasting insecticide nets (LLINs), or in indoor residual spraying. Widespread and long-term use of these insecticides in public health and agriculture has, however, caused a selection and spread of resistance in mosquito populations (Corbel et al. 2007, Nauen 2007, Protopopoff et al. 2008). Recent studies have shown high levels of insecticide resistance in various parts of Africa (Hargreaves et al. 2000, N′Guessan et al. 2007, Sharp et al. 2007, Ranson et al. 2009, Balkew et al. 2010). Because there are only four classes of insecticide approved for use in public health interventions, and only pyrethroids for impregnation of bednets, increasing incidences of (cross-)resistance to these classes are considered a serious threat for the sustainability of contemporary malaria vector control methods (Brogdon and McAllister 1998, Nauen 2007, Kelly-Hope et al. 2008).

The development and use of alternative and sustainable mosquito control agents are, therefore, becoming increasingly important. A novel approach currently being researched is the use of biopesticides containing spores of fungal entomopathogens such as *Metarhizium anisopliae* and *Beauveria bassiana.* Like conventional insecticides, fungal spores act via contact, with spores penetrating the cuticle and proliferating in the hemocoel, typically killing the mosquito in 4–10 d, depending on exposure dose, viability, and virulence of the fungal strain (Blanford et al. 2009, Mnyone et al. 2009a, Farenhorst and Knols 2010). Although clearly slower than the current fast-acting chemical neurotoxins, this life shortening is predicted to reduce malaria transmission, because it takes about 2 wk after a malaria-infected blood meal for mosquitoes to become infectious (Scholte et al. 2005, Hancock et al. 2009, Read et al. 2009). This delayed kill has been suggested to have evolutionary benefits by lowering the selection pressure for resistance development (Thomas and Read 2007, Read et al. 2009).

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Fungal spores can be successfully applied through spraying, dipping, coating, or painting suspensions on several substrate types, including cotton cloth, clay, and paper (Scholte et al. 2005, Farenhorst et al. 2008, Bell et al. 2009, Blanford et al. 2009, Mnyone et al. 2009b, Farenhorst and Knols 2010). Apart from killing the mosquito, fungi can reduce parasite transmission by inhibiting blood feeding (Scholte et al. 2006) and blocking the development of *Plasmodium* parasites (Blanford et al. 2005). Furthermore, fungal biopesticides have potential for integration with existing control tools (Hancock 2009), because fungi are effective against insecticide-resistant anophelines (Farenhorst et al. 2009, Kikankie et al. 2010), and certain insecticide-fungus combinations show synergistic effects on mortality (Farenhorst et al. 2010).

One of the remaining challenges for practical use of fungi for malaria control is the development of delivery systems that maximize mosquito infection rate, enhance spore persistence, and can be integrated into existing control strategies. Application of fungal spores on shaded sites that are attractive for resting anophelines, such as indoor ceiling cloths (Scholte et al. 2005) or clay water storage pots (Farenhorst et al. 2008), can provide effective field delivery options. Mosquito resting behavior can, however, vary between species and environments (Githeko et al. 1996, Mahande et al. 2007) and be further influenced by repellent effects of certain insecticides (Roberts et al. 2000, Pates and Curtis 2005). A complementary approach, therefore, might be to target host-seeking mosquitoes.

One possible strategy would be to target mosquitoes as they enter houses. Doors, windows, and eaves are main points of house entry for anophelines, and screening of these entry routes can offer successful malaria prevention (Lindsay et al. 2003, Kirby et al. 2009). Fungal application on house screens, such as eave curtains, therefore, may provide a suitable delivery tool for infecting host-seeking mosquitoes. To enable the development of such novel systems, it is essential to test whether application of spores on netting material can result in successful infection of mosquitoes.

The aim of the current study was to explore the potential of fungus-treated netting to infect malaria vectors, specifically considering the infectivity and virulence of *B. bassiana* against female *Anopheles gambiae s.s.* mosquitoes. Effects of formulation and application method were assessed and used to evaluate different spore doses and exposure times. Fungal viability and infectivity were tested for three different net types, made from polyester or cotton fibers. Small- and large-meshed polyester nets were compared to assess effects of mesh size and resultant type of contact on fungal efficacy.

Materials and Methods

Mosquitoes. Experiments used 3- to 6-d-old female *An. gambiae s.s.* mosquitoes, which originated from Suakoko, Liberia (courtesy of M. Coluzzi), and were maintained as a colony in the Laboratory of Entomology (Wageningen, The Netherlands) since 1989. Larvae were reared in plastic trays filled with tap water at densities of ≈0.3 larvae/cm^2 and fed on Tetramin fish food (Tetra, Melle, Germany) daily with 1 mg/larva for the first instars and 0.3 mg/larva for the other three larval stages. Pupae were collected daily and transferred to holding cages of 30 × 30 × 30 cm. Emerging adults were kept in climate-controlled rooms (27 ± 1°C, 80 ± 10% RH) and provided with 6% (wt:vol) glucose/water solution.

Fungus. The hyphomycete *B. bassiana* Vuillemin isolate IMI 391510 was used, of which spores (conidia) were produced by solid-state fermentation on glucose-impregnated hemp in aerated packed bed systems (courtesy M. Jumbe, Wageningen University, Wageningen, The Netherlands). After a standard 10-d growth period, spores were harvested through sieving, dried at ambient temperature (to a moisture content <5%), and subsequently stored in 50-ml blue cap tubes in the refrigerator at 4°C. For application, spores were suspended in Ondina 917 oil (Shell Ondina Oil 917, Shell, The Netherlands) or Shellsol T solvent (Shell Shellsol T, Shell, The Netherlands) and mixed through vortexing and sonication at 1000 Hz for 10–15 s (Branson sonifier B12, G. Heinemann, Schwäbisch Gmund, Germany). Spore concentrations of each stock solution were quantified with a Bürker-Türk hemocyte counter (W. Schreck, Hofheim/TS, Germany) using a light microscope at ×400 magnification. Conidial viability was assessed on Sabouraud dextrose agar plates enriched with 0.001% Benomyl and incubated at 27°C for 22–26 h, by counting the proportion of germinated spores under a light microscope (×400 magnification). Stocks showing >85% viability were used for experiments.

Netting. Three net types, two polyester and one cotton, were tested as substrate for fungal applications (Fig. 1). Polyester textile (PT) netting was produced by the Dutch company Van Heek Textiles B.V. (Losser, The Netherlands) and consisted of 150 denier square-knitted multifilament polyester fibers. Two PT mesh sizes were tested, a small-meshed net with 56 holes/cm^2 and a large-meshed net with 15 holes/cm^2. Polyester bednet (PB) material from Vestergaard Frandsen (Lausanne, Switzerland) consisted of 3-mm-thick 100% multifilament polyester fibers, warp knitted in a round-meshed 150 denier net. Two PB mesh sizes were tested, a small-meshed net with 28 holes/cm^2 and a customized large-meshed net with 12 holes/cm^2. Cotton bednet (CB) material from the Dutch bednet supplier Klamboewinkel B.V. (Groningen, The Netherlands) consisted of 100 denier square-knitted fibers of 100% cotton, with a small mesh size of 81 holes/cm^2. Large-meshed cotton netting was not available. For net type experiments, netting pieces were hand washed in 5 liters tap water with ≈5% detergent and 1% bleach and subsequently rinsed five times in tap water.

Application. Pieces of netting (15 × 25 cm) were treated with 5 or 10 ml of *B. bassiana* suspensions of concentrations ranging between 10^{10} and 10^{11} spores/

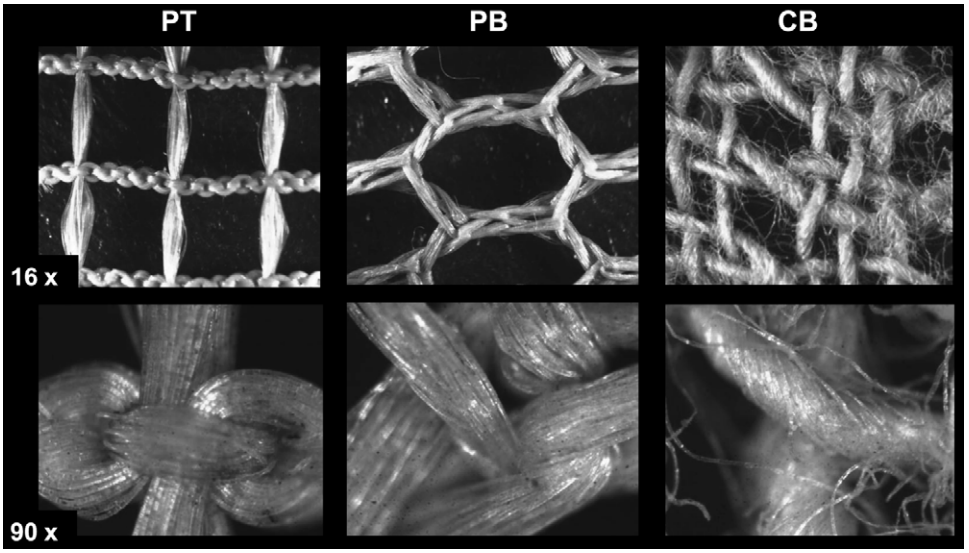

Fig. 1. Photos of PT, PB, and CB substrates at ×16 (top) and ×90 magnification (bottom).

ml, as indicated below. Spray applications used a SATA minijet four high volume, low pressure (HVLP) spray gun (vd Belt, Almere, The Netherlands) at a constant pressure of 1.5 bars. Net samples were attached to a 1-m^2 vertical spray zone within a laminar-flow hood and sprayed evenly at a distance of 30 cm from the samples. Dip application was performed by submerging a piece of net in fungal formulation, manually spreading the suspension over the netting, and subsequently drying the nets horizontally on a wire frame. All net samples were impregnated 1 d before experiments and were dried overnight in a climate-controlled room (27 ± 1°C, 80 ± 10% RH). For controls, net samples were treated with the solvent only.

Infection Assays. Impregnated nets were placed inside a polyvinyl chloride (PVC) tube of 15 cm length and 8 cm diameter, covering the entire inside surface. Both ends were sealed with plastic microwave foil before releasing 40 *An. gambiae* females in each tube via aspiration (Farenhorst and Knols 2010). After exposure, mosquitoes were transferred to plastic holding buckets of 20 cm diameter and 25 cm height via free flight. Mosquitoes that died within 24 h, because of handling, were removed. Holding cages were maintained in a climate-controlled room (27 ± 1°C, 80 ± 10% RH), and mosquito survival was monitored daily. Dead mosquitoes were removed, dipped in 70% ethanol, and incubated 3–5 d on moist filter paper in a sealed petri dish at 27 ± 1°C, after which emerging hyphae were detected using a dissection microscope to verify fungal infection.

Contact Assays. A contact assay was constructed to simulate an eave curtain and mimic the type of contact mosquitoes attempting to pass through impregnated netting would experience. A 20 × 20-cm piece of netting was placed in the center of two plastic cylinders (25 cm long, 15 cm diameter), which were closed off on each end with gauze. Experiments took place in a climate-controlled room (27 ± 1°C, 80 ± 10% RH) under red light conditions at the simulated time of dawn, as this time point corresponded with morning hours and was sufficient to give active and responsive mosquitoes for these laboratory evaluations. Experiments used 40 female mosquitoes per replicate, which were selected after a strong response to human odor the previous day and deprived of sugar overnight. Mosquitoes were released in the left cylinder and attracted to the opposite side by a human hand placed behind the gauze. Mosquitoes were given 30 min to try and cross the netting in the center, after which proportions in both cylinders were recorded, the setup was dismantled, and groups in both cylinders were transferred to separate holding cages via free flight. Mosquito survival was monitored daily. After death, body size was determined by measuring the length of the right wing under the binocular (to the nearest 0.01 mm) (Briegel 1990), and cadavers were placed on moist filter paper to verify fungal infection, as described above.

Experiments.

Formulation. Effects of formulation were tested by comparing fungal efficacy of Ondina and Shellsol suspensions on large-meshed (12 holes/cm^2) PBs. Net samples were sprayed with 5 ml of 10^{11} spores/ml *Beauveria* suspension or solvent only (controls). Survival data were obtained from three replicate groups of 40 female mosquitoes exposed to the nets for 30 min.

Application Method. Effects of application method, i.e., spraying or dipping, were tested on large-meshed and small-meshed PB and PT. Net samples were sprayed with or dipped in 5 ml of 10^{11} spores/ml *Beauveria*-Shellsol suspension or Shellsol only (controls). Mosquito survival data were obtained from four replicate groups of 40 females exposed to the nets for 30 min.

Net Type. Effects of net type on *B. bassiana* efficacy were tested using washed pieces of small-meshed PT, PB, and CB material (Fig. 1). Net samples were sprayed with 5 ml of 10^{11} spores/ml *Beauveria*-Shellsol formulation or Shellsol only (for controls) and kept under climate-controlled conditions (27 ± 1°C, 80 ± 10% RH). For viability tests, proofing paper (Farenhorst and Knols 2010) was also sprayed and used as the positive control substrate. Spore germination rates were measured 1 d, and 1, 2, and 4 wk after application from 1 × 1-cm substrate pieces incubated on sabouraud dextrose agar (SDA), as described above. Spore infectivity was measured 1 d after application on PT, PB, and CB netting by exposing three replicate groups of 40 females for 5 min, resembling potentially short mosquito contact in field settings. PB sprayed with 5 ml of Shellsol was used as control substrate.

Contact Type. Effects of contact type, i.e., passage or nonpassage through netting in the contact setup, were tested using large (12 holes/cm^2)- and small-meshed (28 holes/cm^2) PB material sprayed with 5 or 10 ml of 5×10^{10} *Beauveria* spores/ml (or Shellsol only for controls). Three replicate groups of 40 females were released in the setup for 30 min. Infection and survival rates were compared for groups that had passed though the net with groups that had not.

Data Analysis. Efficacy of fungus-impregnated netting was analyzed using the cumulative daily proportional mosquito survival after exposure. To measure the impact of different fungal treatments on mosquito survival, Cox regression survival analyses (SPSS 16.0, Chicago, IL) were used, which compute hazard functions that quantify the instantaneous risk of death at each time point. Differences in overall mortality rates (over the whole survival curve) were given in hazard ratio (HR) values. A HR value of 1 indicated an equal average daily risk of dying in both tested groups, a HR <1 lower, and HR >1 higher overall mortality in group 2 compared with group 1, respectively. The 95% confidence intervals (95% CI) were provided for each computed HR. Plots of survivor functions were used to check HR proportionality. Effects of solvent, net type, and contact type on fungal impact were assessed by measuring significant interactions between the test factors and fungal impact with a full Cox regression model (all covariates and interactions included). For contact assays, differences in mosquito proportions passing through the net and body size were compared used an independent sample *t* test in SPSS 16.0. A significance level of <0.05 was used in all analyses.

Results

Formulation and Application. All fungus-impregnated netting samples induced significant reductions in mosquito survival ($P < 0.01$) (Fig. 2). The onset of fungal impact was observed around 3 d postexposure, which is consistent with the time point in which these hyphomycetous fungi are known to start proliferating within the insect and to approach their exponential growth phase (see Bell et al. 2009). *Beauveria* spores had a twice as high overall impact on mosquito survival when suspended in Shellsol compared with Ondina (HR = 2.12, 95% CI = 1.83–2.70, $P < 0.001$), suggesting some benefit of a lighter, more evaporative oil. All samples appeared sufficiently dry before use in exposure tests, and a longer drying period of 3 d did not affect fungal efficacy (data not shown). Shellsol T was, therefore, chosen as the standard formulation for subsequent netting applications.

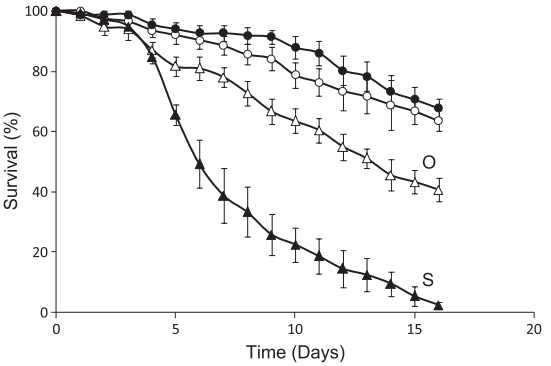

Fig. 2. Effect of formulation on fungal efficacy. Impact of *B. bassiana* formulations on *An. gambiae s.s.* survival (mean ± SEM) after spray application on PB polyester nets (using 5 ml of 10^{11} spores/ml). ○, Represent nets sprayed with Ondina oil; ●, show nets sprayed with Shellsol solvent; △, represent nets sprayed with *Beauveria* spores dissolved in Ondina; and ▲, show nets sprayed with *Beauveria* dissolved in Shellsol.

Spraying resulted in visibly lower concentrations of fungal spores adhering to netting substrates than dipping. Interestingly, however, spraying resulted in higher fungus-induced mortality rates (Fig. 3), with significant interactions in the effect of fungus (compared with corresponding control mortalities) and application method for all net types ($P < 0.05$), except for small-meshed PB (28 holes/cm^2, $P = 0.08$).

Netting mesh size did not influence fungal efficacy in the tube exposure assay, as there were no significant interactions between fungal impact and mesh size for either net type. PB induced between 16 and 25% higher average mortality at day 14 compared with PT (Fig. 3). Impact of *Beauveria* on PB was higher in sprayed samples, showing significant interactions between fungus and net type for sprayed small-meshed ($P < 0.001$) and large-meshed nets ($P = 0.003$). Fungal efficacy was not higher on PB substrates when nets were dipped in fungal formulation (Fig. 3).

Net Type. Effects of substrate on fungal efficacy were further explored using three different net types (Fig. 1). Spores from the same suspension were less viable after application on polyester compared with cotton or paper (Table 1). There was a significant drop (≈20%) in spore viability on PB and PT immediately after application. Cotton fibers, however, did not reduce spore viability compared with the positive control substrate (Table 1). There were modest differences in the longer-term viability of spores between net types, with germination rates decreasing by 32%

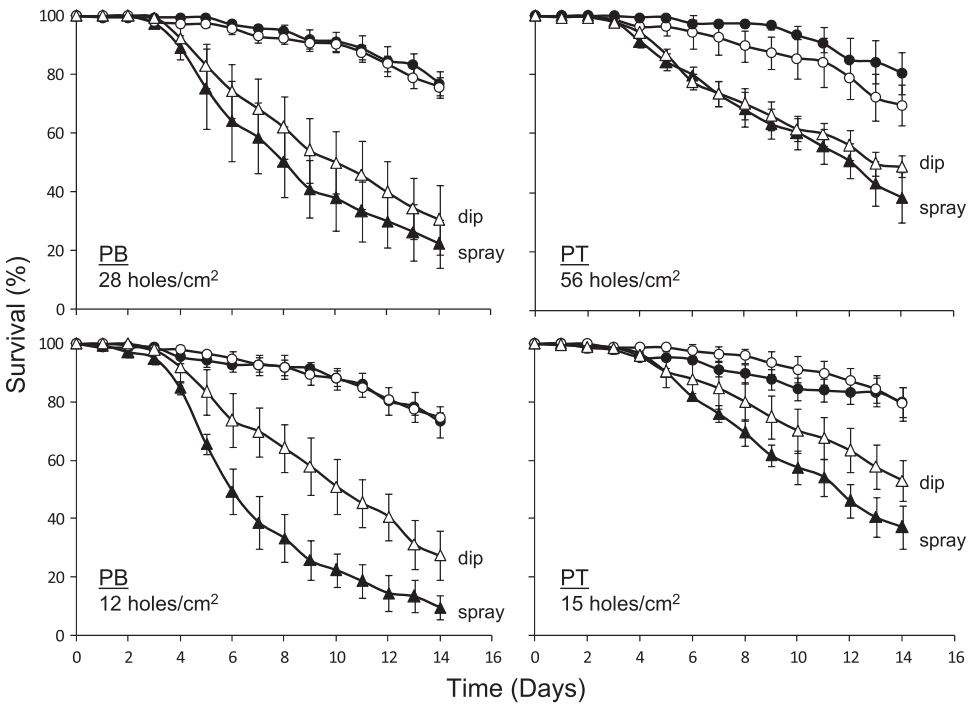

Fig. 3. Effects of application method, substrate type, and mesh size. Impact of *B. bassiana* formulations on *An. gambiae s.s.* survival (mean ± SEM) after spray application on PB or PT substrates with small (top) or large mesh sizes (bottom). Triangles represent nets treated with *Beauveria* formulation, and circles represent control nets treated with Shellsol only. Treatments were applied using spray application (solid symbols) or dipping the nets (open symbols).

on PT, 22% on PB, 21% on CB, and 18% on paper substrates within 4 wk after application (Table 1).

Exposure tests showed that all three net types were effective *Beauveria* spore carriers, inducing significant reductions in *An. gambiae* longevity after 5-min exposures (Fig. 4). Despite differences in viability, the applied spore dose induced similar mosquito mortality on PB and CB substrates ($P > 0.05$). Fungal impact on mosquito survival was, however, almost twice as high on bednet material (PB) than on PT (HR = 1.88, 95% CI = 1.21–2.78, $P = 0.019$), indicating that PT was a less suitable substrate for mosquito-fungus infections.

Contact Type and Mesh Size. On average, 48% (range, 40–58%) of mosquitoes passed through the 12 holes/cm^2 PB net in the exposure cylinders within half an hour. Wing size was not significantly different between groups that passed through the net (2.7 ± 0.5 mm) and those that did not (2.8 ± 0.7 mm) (T = 0.99, df = 463, $P = 0.319$), indicating that body size did not influence the mosquito's capability to cross the net. Duration of contact differed between individuals, although <5% of mosquitoes were not enticed to contact the net at least once.

Both fungal concentrations induced significant reductions in mosquito survival regardless of contact type, killing 100% female *An. gambiae* within 16 d (Fig. 5) with >85% showing fungal sporulation after death. The higher spore concentration (5×10^{11} spores) had a significantly greater impact on mosquitoes that had

Table 1. Average ($n = 3$) *Beauveria bassiana* spore germination rates measured 1 d, and 1, 2, and 4 wk after spray application on netting samples of PT, PB, CB, or paper

Net type	Material	Spore viability (germination %)			
		1 d	1 wk	2 wk	4 wk
PT	Polyester	75	60	47	43
PB	Polyester	71	63	50	49
CB	Cotton	91	86	76	68
Positive control	Paper	92	90	83	74

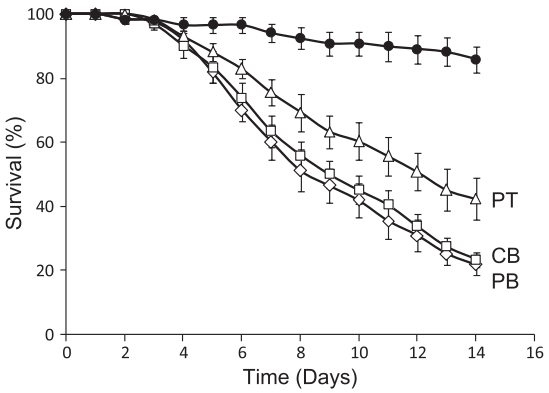

Fig. 4. Efficacy of different net types. Mean (±SEM) mosquito survival after 5-min exposure to small-meshed PT, PB, or CB material sprayed with *B. bassiana* (5 ml of 10^{11} spores/ml), or a control PB net treated with solvent only (●).

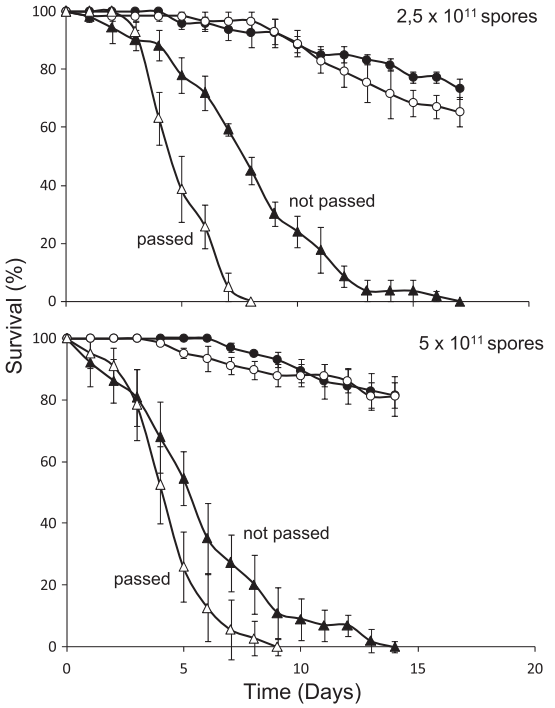

Fig. 5. Effects of mosquito passage. Mean (±SEM) survival of *An. gambiae s.s.* after superficial contact with (not passed) or passage through (passed) PB netting that was spray impregnated with 2.5×10^{11} (top) or 5×10^{11} (bottom) *B. bassiana* spores (triangles) or solvent only (circles). ○, Represent control nets sprayed with Shellsol only through which mosquitoes passed; ●, show control nets through which mosquitoes did not pass. △, Represent nets sprayed with *Beauveria* spores dissolved in Shellsol through which mosquitoes passed; ▲, show fungus-treated nets through which mosquitoes did not pass.

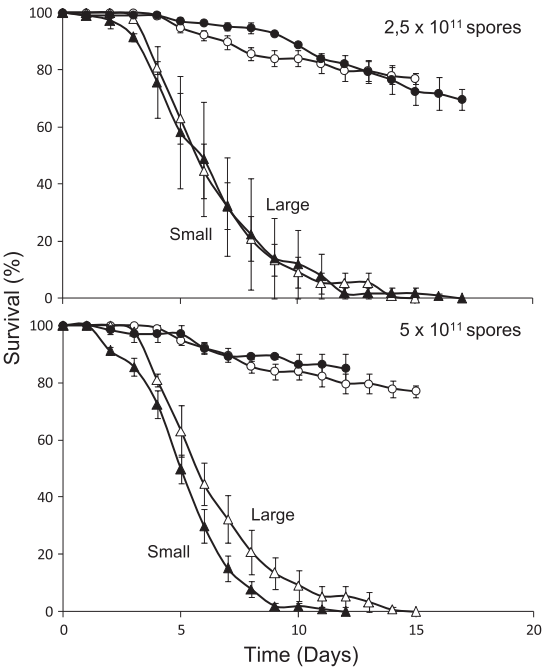

Fig. 6. Effects of mesh size on netting efficacy. Impact of 2.5×10^{11} (top) or 5×10^{11} *B. bassiana* spores sprayed on large-meshed (Large) or small-meshed (Small) PB netting on *An. gambiae s.s.* survival (mean ± SEM) after 30-min exposure in the contact assay. ○, Represent large-meshed control nets sprayed with Shellsol only; ●, show small-meshed control nets. △, Represent large-meshed nets sprayed with *Beauveria* spores dissolved in Shellsol; ▲, show small-meshed fungus-treated nets.

not crossed the net compared with the lower concentration (HR = 1.83, 95% CI = 1.23–2.71, $P = 0.003$). There was no effect of spore concentration in groups that passed through the netting (HR = 1.05, 95% CI = 0.81–1.69, $P = 0.4$). Fungal impact on survival was significantly higher in groups that passed through netting compared with groups that did not (Fig. 5), for both the low (HR = 3.73, 95% CI = 2.25–6.12, $P < 0.001$) and high spore concentration (HR = 1.62, 95% CI = 1.13–2.33, $P = 0.009$). The small-holed PB net (28 holes/cm^2) that blocked all mosquitoes from passage was included in the contact experiments to measure the impact of mesh size on the infection efficacy. Passed and not passed mosquito data from the large-mesh treatment were pooled to compare overall impact on survival. Large- and small-meshed netting were both suitable spore carriers, enabling effective *Beauveria* infections with >90% mortality within 10 d (Fig. 6). On large-meshed nets, there was no significant effect of spore concentration, whereas on small-meshed nets the high concentration was significantly more effective (HR = 1.23, 95% CI = 1.09–1.40, $P = 0.001$).

Discussion

This study demonstrates that netting materials can be used to deliver lethal doses of fungal spores to adult mosquitoes. The results of the specific experiments reveal a number of factors that are likely to be important in any further development of the approach. First, the nature of the oil formulation was shown to make a difference, with a light evaporative oil (Shellsol) producing more rapid and extensive mortality than a heavier, viscous oil (Ondina). The reasons for this are unclear, but it is possible that the thicker oil caused stronger adherence of spores to the netting, reducing transfer to mosquitoes. This effect was also observed in a study evaluating coating applications on paper (Farenhorst and Knols 2010).

Second, fungal sprays were more effective than dipping applications. This was a slightly surprising result as netting represents a very open target for spraying (i.e., there are many gaps through which spray droplets can pass, which is likely why small-meshed nets were more effective), and thus, spraying results in a reduced effective spore concentration per unit area. However, it is possible that submerging the netting resulted in spore aggregations and/or high absorbance of spore suspension on or into fibers, whereas spraying may have given a finer distribution of spores on the

outer surface that induced better transfer to mosquitoes. Further studies on spore retention and distribution could be informed by methods such as quantitative polymerase chain reaction (Bell et al. 2009).

Third, efficacy varied between the different net types, with PT significantly less effective than cotton in terms of fungal impact on mortality and spore persistence. Because PT has smooth fibers, part of this effect could result from poor spore attachment to the netting material. However, the accelerated loss in spore viability over time for both polyester materials tested suggests some sort of chemical effect, either from the polyester itself or from added chemicals such as phthalates, which are used to soften polyester fibers (Pang et al. 2006). Although cotton has been successfully used in bednets and eave curtain nets (Majori et al. 1987), polyester is the most widely used material for bednets and house screens because its strong fibers are more durable, give more ventilation, and retain insecticide better (Curtis et al. 1996, WHO 2001). Thus, further research is required on the chemical composition of polyester and potential reactions with fungal or solvent compounds to better understand the effects on spore viability. Such research should also consider effects of polyolefin polymers that are now being used within insecticide-treated net (ITN) fibers (Kilian et al. 2008). Other studies have shown that *Beauveria* strains can have a residual activity of several months under laboratory conditions (Darbro and Thomas 2009). However, the absolute long-term spore persistence, i.e., after application, will need to be further evaluated under realistic environmental conditions.

Finally, infection rates were highest in mosquitoes that were able to pass through impregnated netting, which was most noticeable for lower spore doses, suggesting greater physical transfer of spores as the mosquitoes traverse the net. However, overall fungal efficacy was not dependent on mesh size or the ability of mosquitoes to penetrate the netting. These results have interesting implications for different application strategies. Use of small-meshed, fungus-impregnated eave or window screens would infect mosquitoes as they land on the netting and attempt to probe. One concern with eave curtains, however, is the effect they have on airflow, and it has been suggested that larger mesh sizes might be more desirable (Majori et al. 1987, Hossain and Curtis 1989). Large-meshed fungus-impregnated eave nets would allow for more airflow while effectively infecting mosquitoes that traverse the net. This would not give immediate personal protection, because fungi take time to kill, but could deliver community-wide benefits by reducing the abundance of old, potentially infectious mosquitoes (Hancock 2009, Read et al. 2009). For field use, however, such an approach will likely only be acceptable when used in combination with interventions that provide personal protection.

Another possible delivery method would be to follow the ITN model and apply fungal spores directly onto bednets. However, whereas insect-pathogenic fungi such as *B. bassiana* and *M. anisopliae* generally pose negligible risk to human health and the environment (Zimmermann 2007a, b; Darbro and Thomas 2009), the use of spores on bednets would clearly need to be subjected to rigorous safety testing before such interventions will be acceptable. Additionally, long-term viability of spores on bednets might be compromised by regular washing (although whether this could be overcome by novel formulation or impregnation techniques is not known). Novel techniques in bednet development may, however, facilitate use of fungal spores. For instance, two-in-one combination bednets are being developed, which use a slow-acting, nonirritant chemical insecticide on the apex or roof of the net and fast-killing pyrethroids on the sides (Guillet et al. 2001, Oxborough et al. 2008). Fungal spores may potentially be used as the slow-acting pesticide on the tops of such nets, away from human contact, with repellent pyrethroids on the sides preventing blood feeding and providing personal protection.

Delivery of fungal spores on house screens can, nevertheless, be considered more viable for field implementation (in terms of acceptability and long-term efficacy because of less handling) and could provide options to integrate fungal biopesticides in contemporary chemical malaria vector control measures. Combined implementations have shown promise in previous studies (Farenhorst et al. 2009, Hancock 2009) and could, for instance, be achieved by adding fungi onto insecticide-treated eave curtain nets. The widely used insecticide permethrin, however, acts as a contact irritant, which may lead to shorter resting times or reduced pickup of fungi if the two were presented together on a combination net. Combined interventions could also be spatially separated by, for example, using fungus-impregnated house screens together with LLINs, which would still allow for coexposure of mosquitoes to both agents in a single feeding episode. Overall, results indicate that the use of biological control with fungal spores dissolved in Shellsol and sprayed on small-meshed cotton eave curtain nets would be the most promising option for field implementation with potential for integration into chemical malaria vector control with LLINs or indoor residual spraying. When used in integrated vector management strategies, fungal biopesticides could provide a means to target the resistant fraction of the mosquito population, which could not only help to sustain malaria control, but also to slow the spread of resistant alleles in the mosquito population.

This laboratory study has explored the potential efficacy of fungal applications on netting and showed that spray applications of fungal solutions on several netting substrates can be highly effective against anophelines. Although further optimization is required to realize field deployment, fungus-impregnated house screens and barriers could provide a means to target host-seeking mosquitoes and maximize the impact of fungal biopesticides on malaria transmission in field settings where insecticide resistance is a growing threat to contemporary malaria interventions.

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