FUNGAL ENTOMOPATHOGENS FOR ADULT MOSQUITO CONTROL – A LOOK AT THE PROSPECTS

Bart Knols & Matt Thomas report on a Consortium Workshop held in South Africa on the potential use of entomopathogenic fungi as biopesticides to control adult mosquitoes vectoring human diseases.

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All methods currently deployed in tropical regions against adult mosquito populations that transmit debilitating diseases such as malaria and dengue are based on insecticide application. For instance, the uptake and use of insecticide-treated bednets (ITNs) for personal protection has been dramatic in many developing countries (e.g. Vietnam; see WHO, 2002) and continues to rise (WHO, 2005). DDT, used for indoor residual spraying (IRS), narrowly escaped the Persistant Organic Pollutants treaty a few years ago and has since gained in popularity in various African countries – it is cheap and it works (Curtis, 2002). In as much as these insecticide-based strategies provide huge public health benefits, either at the personal or community level, widespread use inevitably leads to vector resistance and the resulting problems to manage it (Hemingway, 2004). A few years ago, several research groups discovered independently that the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*, which have a history of use for control of agricultural pests in various parts of the world (including, for example, a *Metarhizium*-based biopesticide produced and registered for control of locusts and grasshoppers in Africa), can also infect and kill adult mosquitoes. Since 2005, these groups have joined forces and met in Johannesburg in March to discuss the status of this novel approach, critical scientific issues and the prospect and requirements for developing this strategy to operational levels in disease-endemic countries, particularly those affected severely by insecticide resistance and associated problems.

Current status

The use of entomopathogenic fungi against mosquitoes is not a new idea (for a comprehensive review on the topic see Scholte *et al*., 2004) yet their widespread use (mostly against the aquatic stages of mosquitoes) has been curtailed by limited persistence after application together with production and storage/shelf-life difficulties. The Consortium acknowledges these difficulties, but considers its recent studies of sufficient value to reconsider the application of fungi for *adult* mosquito control (Fig. 1). First, using a rodent malaria model, Simon Blanford and colleagues (from Edinburgh University and Imperial college) found that infections with *B. bassiana* not only had a dramatic impact on mosquito survival, but also impacted on the malaria parasite within infected mosquitoes, resulting in an 80-fold reduction in the transmission potential of *Plasmodium*-infected *Anopheles stephensi* (Blanford *et al*., 2005). Second, Ernst-Jan Scholte and colleagues at Wageningen University and the Ifakara Health Research and Development Centre (Tanzania) demonstrated that *M. anisopliae*, when applied as spores in oil formulation on cloth suspended in rural Tanzanian houses, was capable of infecting nearly a quarter of the mosquitoes resting on the cloth. Using an entomological inoculation rate model, it was estimated that even at this moderate coverage the impact on malaria transmission could be substantial (Scholte *et al*., 2005). Both of the above groups also reported a reduction in blood-feeding propensity of fungus-infected mosquitoes, further reducing the likelihood of pathogen transmission (Scholte *et al*., 2006). Third, laboratory studies by Jenny Stevenson at the London School of Hygiene and Tropical Medicine have delivered promising results of these fungi against a multiple insecticide-resistant strain of *Anopheles stephensi*, fuelling hope that fungal pathogens can be deployed to overcome problems in areas where pyrethroid resistance is widespread (Stevenson *et al*., unpubl. data).

Figure 1. **An *Anopheles gambiae* mosquito, overgrown with the fungus *M. anisopliae* (photo: E-J Scholte).**

Although these developments have been hailed widely (ffrench-Constant, 2005; Kanzok & Jacobs-Lorena, 2006) concerns were also raised (Enserink, 2005; Michalakis & Reynaud, 2005; Ward & Selgrade, 2005; Hutchinson & Cunningham, 2005). These can be grouped into issues related to mode of action and the mosquito-*Plasmodium*-

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fungus interactions, impact of fungal spores on human and enviromental health, and practical concerns related to field application (Thomas *et al*., 2005). Below we elaborate on these issues identifying some of the ongoing activities and approaches currently being addressed within the consortium.

The Consortium on entomopathogenic fungi for the control of adult mosquitoes currently consists of seven partner institutions (CSIRO, Australia; IHRDC, Tanzania; Univ. of Durham, UK; NICD, South Africa; LSHTM, UK; Univ. of Edinburgh, UK and Wageningen Univ., The Netherlands). Since 2005, its mission is to reduce the burden of mosquito-borne disease through the development and delivery of novel and sustainable approaches of adult vector control based on fungi. The Consortium seeks to maximise scientific progress of its individual partners and the Consortium overall, maximise synergies and minimise overlaps, and capitalise on the advantages of collective interests and collaborative partnerships.

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Critical issues

Unlike chemical insecticides, for which the only aim is to kill mosquitoes rapidly following contact, fungal biopesticides present more complexity with regard to fungal isolate choice, delivery options and persistence (Fig. 2).

- *Isolate characteristics and effects*. Fungal isolates will vary in terms of infectivity (i.e. the ability to infect a mosquito) and virulence (i.e. time required before desirable effects take place). At present, little is known about the processes that govern fungal infectivity to adult mosquitoes or the range of lethal and/or sub-lethal effects that can result. While we have already identified a number of highly promising isolates with interesting infection phenotypes, only a relatively limited range of isolates have been screened within the consortium to date. One of the key ongoing activities, therefore, is to conduct more extensive screening, evaluating isolates from three broad perspectives: (i) virulence of isolates and ability to cause rapid mosquito kill (essentially similar to a chemical insecticide); (ii) ability of fungal isolates to alter parasite development in the mosquito (rendering it a 'transmission-blocking biopesticide'); (iii) isolates that yield anti-transmission effects, for instance by influencing the mosquito's host-seeking behaviour and/or reduction of its feeding propensity. Each of these options (or combinations thereof) will exert some evolutionary pressure on the mosquito and possibly malaria itself. Our intention is to factor the evolutionary consequences into our thinking at an early stage as part of the basis for rational selection of isolates. For instance, delayed kill of mosquitoes is assumed to reduce the likelihood of anti-fungal resistance development, as females will have (at least partial) reproductive success through production of one or more batches of eggs before succumbing to the infection. Hence, although possibly counter-intuitive, isolates causing relatively late acting mortality might be preferable to highly virulent isolates.

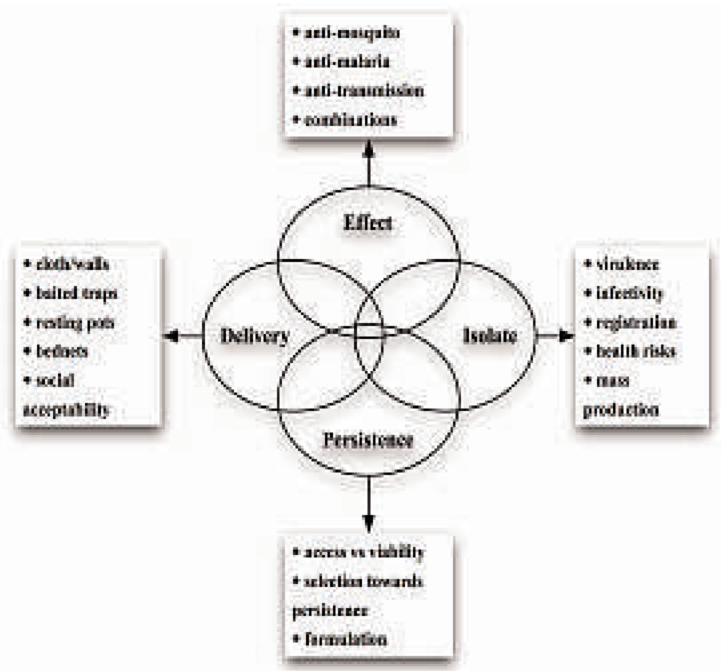

Figure 2. **Complexity of choice in the selection of suitable fungal isolates for control of adult mosquitoes.**

- *Human and environmental health concerns*. Beyond biological prerequisites, isolate choice will be affected by possible health risks posed by their application in domestic settings. Concerns have been raised over the possible impact of spore inhalation by humans, particularly in regions where other diseases (notably HIV/AIDS) affect the immunocompetence of its population (Ward & Selgrade, 2005; Hutchinson & Cunningham, 2005). Although, for various reasons (e.g. application of spores in oil formulation) this risk is currently considered low, the Consortium acknowledges this concern and intends to research this through partnership with medical mycologists. As for non-target organism effects, application of fungal spores in the indoor environment is likely to affect other nuisance insects or vectors of pathogens, depending on the host-range of the isolate used. Such effects will be researched from a cost-health benefit perspective and compared with environmental effects posed by contemporary insecticide-based strategies.

- *Delivery and persistence*. Given a suitable isolate that poses minimal health risks, two key challenges remain. First, optimal means (both in efficacy and economic terms) to deliver the fungus to mosquitoes need to be developed. Application of the spores inside houses, where many malaria and dengue vector species prefer to blood-feed and rest optimises the likelihood of fungus contact and infection. Moreover, traditional houses are generally cooler and more humid than their surroundings, and spores will be protected from exposure to sunlight, all of which will enhance fungal persistence. This latter characteristic is likely to prove critical in furthering the development of fungal biopesticides. Persistence (i.e. the ability to infect mosquitoes over time) is of course an innate characteristic of fungal spores, and improved understanding of the factors influencing this trait is likely to yield, or enable selection for, isolates that enable frequency of re-application to be reduced. In addition, persistence will be strongly affected by the nature of the formulation and substrate treated. Research in this area is considered a priority by the Consortium.

Beyond isolate choice and its formulation, actual delivery to the mosquito is another field of research. Our field trials so far have used partial indoor treatment of spores on cloth (Scholte *et al*., 2005) but novel applications (e.g. on bednets or in artificial mosquito refuges) need to be researched. Minimal application (to reduce cost and health risks) and maximum exposure/viability is the ultimate goal of these studies.

Towards field application

The above issues represent key research areas that Consortium partners will focus on over the next few years. Yet, bridging the divide between laboratory research and operational field implementation will pose additional challenges. First, even though models have shown that fungal application may lead to substantial reduction in malaria transmission, this needs verification in the real world. Similar to the large-scale WHO-funded trials in the mid-1990s, to evaluate the benefits of ITN usage in terms of morbidity and mortality reduction, fungus-based strategies will only gain acceptance if unambiguous public health benefits are demonstrated. To this end, the ground is being prepared for a large-scale cross-over trial involving 12 villages in southern Tanzania, with the first treatments planned for 2008. Beyond establising the potential level of public health gains, it needs to be ascertained how the technology will ultimately be utilised. At this stage it seems more likely that it will be implemented programmatically through existing entities in charge of vector control. However, the nature of the product (i.e. it is not a chemical) and likely delivery mechanisms (such as impregnated cloths) will require active engagement of end-users early on to develop appropriate understanding and capacity for adoption. In addition, the implementation strategy will also be influenced by the scale and scope at which fungal mass production is economical. Throughout the world mass production facilities for fungal entomopathogens vary from small village-scale systems, through semi-commercial production to large-scale commercial facilities servicing national or regional markets. The appropriateness of these different models depends on supply and demand (including issues around economies of scale), technical considerations (including issues of quality control, storage and formulation capacity, distribution networks) and also regulatory factors such as product registration (including the ownership and costs of developing a registration dossier). These implementation issues are as critical as the technical research and development issues; there is little value in developing a technical solution that ends up sitting on the shelf.

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