Entomopathogenic fungi as the next-generation control agents against malaria mosquitoes

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The massive uptake of bed nets in Africa will mark the end of an era where pyrethroid insecticides were useful. Widespread resistance will likely terminate their role in vector control in the next decade.

The first decade of the new millennium experienced intensified efforts to control malaria, notably in Africa. Due to a massive uptake in the use of insecticide-treated bed nets, indoor residual spraying and improved case management, several countries witnessed a remarkable decline in the number of malaria cases and deaths. The recently published World Malaria Report 2009 reported that funding for malaria control has increased from US$0.3 to 1.7 billion over the last 6 years, that 31% of all African households owned at least one treated bednet in 2008 and that a third of the 108 countries that provided data for the report experienced dramatic reductions in the number of cases [101]. Optimism reigns, with claims that malaria will be eliminated from various parts of the world in the coming decades. During the malaria conference in Nairobi last November, Kenyan health authorities even declared that by 2017 the disease will have been eliminated from its territory, provided sufficient funding is available [102]. A roadmap for malaria elimination entitled ‘Shrinking the Malaria Map’ was published in April 2009 [103], fuelling enthusiasm that malaria will one day follow the same fate as smallpox and be eradicated.

Loss of tools

However, these intensified and concerted efforts will continue to bear fruit only if the current tools remain effective and new ones reach the end of the research and development pipeline quickly enough to replace those that have lost effectiveness. For the only class of insecticides that is currently used to impregnate bed nets, the pyrethroids, the outlook is grim, with high levels of resistance already recorded in anopheline mosquitoes in west and southern Africa. The up-scaling of control efforts across the continent will further aggravate this problem, and a recent report of the WHO/Research and Training in Tropical Diseases (TDR) network on insecticide resistance in African malaria vectors confirms this trend [1]. In response, the search for new chemicals to replace pyrethroids has intensified [2] and may yield alternatives in due course, although a priori any widespread use of a new insecticide will meet the same fate as existing ones – loss of effectiveness due to resistance. The question then becomes: can we be smarter in the way we control mosquitoes and escape the treadmill of insecticide resistance? A closer look at the principles of evolutionary biology suggests we can [3–5].

Evolution-proof control tactics

The reason why vector populations develop resistance to insecticides is the fact that chemicals used on bed nets or sprayed inside houses target the newly emerged adult insects early in life and kill these within a day following exposure. This approach exerts a high selection pressure on mosquitoes, providing huge fitness advantages to resistant mutants and consequently the rapid spread of resistance traits in vector populations. The search for active chemicals that can kill mosquitoes is based on principles that were laid down nearly 40 years ago, and these have not changed since [6]. The aim of these principles is to obtain at least 50% knockdown (or death) of exposed mosquitoes within 24 h following exposure at a concentration of 16 µg active ingredient/cm² [2]. Consequently, this search strategy will continue to yield chemicals that favor the rapid selection for resistance.

However, for malaria mosquitoes there is no need to kill young adults as they are completely harmless. Young females first need to acquire
an infection during a bloodmeal that contains gametocytes and will then need 10–14 days to mature the infection (the so-called extrinsic incubation cycle). It is only from this time onwards that their salivary glands contain sporozoites that can infect humans during subsequent blood meals. In short, malaria mosquitoes are harmless in the first two weeks of life and only a small proportion of the female population lives long enough to transmit parasites. If we could shorten the adult lifespan of females by just a few days, the impact on transmission would be tremendous. For the dengue virus, the story is very similar where virus replication in the mosquito takes time and precedes its ability to transmit the virus.

Using this logic, what would happen in terms of resistance to control tools if we specifically targeted older females, just before they are capable of transmitting disease? First, we would not exert the high selection pressure that insecticides induce. In fact, we would allow females to reproduce and lay two, maybe three, batches of eggs. Second, resistance normally bears a fitness cost, and resistance to any late-life killing chemical or control agent would only be beneficial to older specimens in the population, with the bulk of the (younger) population paying the price. This would naturally limit the spread of any (unlikely) resistance. Andrew Read and colleagues modeled these effects recently and concluded that late-life killing agents could dramatically reduce the risk of resistance developing [4]. In addition, fungal invasion of mosquitoes results in multiple modes of attack, further reducing the likelihood of resistance development.

Several lines of research on control methods that will only kill mosquitoes late in life are underway. The endosymbiont Wolbachia, which occurs naturally in fruit flies, was stably transferred to the dengue vector Aedes aegypti and shortens the adult female lifespan by several days [7]. Entomopathogenic fungi, notably Metarhizium anisopliae and Beauveria bassiana, can infect mosquitoes early in life and kill them, depending on the exposure dose and fungus isolate, after 3–14 days. Both Wolbachia and fungi have also been shown to interfere with dengue virus and Plasmodium parasite development in the mosquito, respectively, with near-complete blockage of virus replication [8] and parasite progression from oocyst to salivary gland invasion by sporozoites [9]. It may even be possible to select for nonvirulent fungal isolates that, after infecting mosquitoes, inhibit Plasmodium development but have no serious impact on mosquito longevity.

What’s next?

Mosquitoes that are resistant to insecticides remain susceptible to fungus infection. A recent study has shown that infection with fungus actually increases the susceptibility of insecticide-resistant mosquitoes to the chemicals they had developed resistance to [10]. Beyond extending the lifespan of currently used insecticides, fungi may offer a good alternative in countries such as Benin, where resistance has reached unacceptable levels. A study that modeled the combined effect of fungal biopesticide application with the use of insecticide-treated bed nets produced favorable scenarios for integrated use of these tools [11]. And in a small-scale field trial in Tanzania, Metarhizium spores applied in sunflower oil formulation on black pieces of cotton cloth on which mosquitoes prefer to rest after feeding infected nearly a quarter of them [12]. Beyond killing adult mosquitoes, our recent work has also demonstrated excellent activity of fungi against the aquatic larval stages, opening up further possible uses of these biopesticides [13]. So what is needed to bring biological control of malaria mosquitoes to the forefront of disease control in Africa and elsewhere? First and foremost, it is essential that large-scale field trials are conducted to demonstrate the effects of fungi not only on mosquitoes but on their potential to reduce disease transmission. Similar to the case of bed nets, which became widely accepted after several large-scale trials in various African countries unequivocally demonstrated their ability to save lives [14], support can only be expected after similar results are obtained for fungi. Adoption of these biopesticides in the WHO pesticide evaluation scheme will be equally important and necessary to grant clearance for wider use and application of this novel technology. On the technical front, several hurdles need to be cleared. At present, the viability, infectivity and persistence of fungal spores under field conditions are inadequate, which necessitates the development of long-lasting formulations that can infect and kill mosquitoes at least 6 months after application. Delivery methods need to be optimized to maximize exposure of mosquitoes both in- and outdoors with minimal usage of spores. And although three countries in Africa (South Africa, Kenya and Senegal) produce fungal spores for agricultural pest control, no industrial-scale production of antimosquito fungi is currently underway. None of these issues (production, formulation, delivery) are
terribly exciting from a biomedical point of view, but they are vital if biopesticides for adult mosquito control are ever to reach the market. Painstaking research conducted over the last decade has delivered solid evidence that fungi can become a next-generation tool to control mosquitoes. Now is the time for an entrepreneurial entity to take over and bridge the gap between the world of science and the world of disease control.

Bibliography


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