An entomopathogenic fungus (*Metarhizium anisopliae*) for control of the adult African malaria vector *Anopheles gambiae*

Ernst-Jan Scholte
Bart G.J. Knols
Willem Takken

KEY WORDS
insect pathogenic fungi, biological control, Diptera, malaria control

Entomologische Berichten 68 (1): 21-26

Current indoor control for malaria vectors in Africa depends on the use of insecticides and/or repellents. Although several biological control agents can reduce larval mosquito populations, none are appropriate for targeting the adult stage. This manuscript describes a series of experiments to evaluate the potential impact of a new method, the use of an insect-pathogenic fungus (*Metarhizium anisopliae*) against adult *Anopheles gambiae* sensu stricto, both in the laboratory and in the field. Various experiments describe different aspects of this developing methodology, among which application techniques, sublethal effects, and the impact it may have on malaria transmission. Finally, it is discussed how this method can play a part in an integrated approach of mosquito vector control in the tropics.

Introduction

For a long time, the principal choice to reduce nuisance biting by mosquitoes or their transmission of parasitic or arboviral diseases has been the selective application of residual synthetic insecticides. The public health benefit delivered by this method, especially in tropical resource-poor settings, is huge, saving uncountable human lives. Powered by a strong industrial lobby, new and more environmentally friendly compounds replace older, more harmful ones. However, beyond gains in economic and public health terms, the grim reality of environmental impact and ever-developing resistance remains an issue of grave concern (Chandre *et al.* 1999, Hemingway & Ranson 2000, Brooke *et al.* 2002). It is therefore not surprising that interest in alternative non-chemical strategies has increased over the last decades. The use of biological control agents such as protozoa (Chapman 1974, Legner 1995), nematodes (Kaya & Gaugler 1993), predatory fish (Legner 1995), and bacteria (Becker & Ascher 1998) has shown promise as a means to control mosquito populations, and some of these agents are successfully used in today's mosquito control programmes in various regions around the world (Becker *et al.* 2003). Unfortunately, in the one continent where malaria accounts for more than 1 million deaths each year, alternatives to insecticides are virtually non-existent. Recently, interest has increased in another group of biological

Fungus	mosquito species	affected stage	reference
Oomycota[1]			
Leptolegnia[2] sp. (unidentified)	*An. gambiae*	larva	Nnakumusana 1986
Pythium sp.	*An. gambiae*	larva	Nnakumusana 1985
Lagenidium giganteum	*An. gambiae*	larva	Golkar *et al.* 1993
Chytridiomycota			
Coelomomyces indicus	*An. arabiensis*	larva	Service 1977
	An. gambiae	larva	Muspratt 1963
Zygomycota			
Smittium (unidentified)	*An. gambiae*	larva	Coluzzi 1966
Fungi Anamorfici			
Aspergillus parasiticus	*An. gambiae*	larva/adult	Nnakumusana 1985
Metarhizium anisopliae	*An. gambiae*	larva/adult	Roberts 1967, Scholte *et al.* 2003
Beauveria bassiana	*An. gambiae*	adult	Scholte *et al.* 2003

[1] = Phylum, [2] = Genus

Table 1. Entomopathogenic fungi that have been reported to be pathogenic to African malaria vectors.
Table 1. Entomopathogene schimmels waarvan gerapporteerd is dat ze pathogeen zijn voor Afrikaanse malariavectoren.

1. *Metarhizium anisopliae*, sporulating from a cadaver of the African malaria mosquito *Anopheles gambiae* s.s. The green-whitish colour is caused by millions of conidia, produced by conidiogenous cells forming on hyphae growing out of the deceased mosquito. Photo: E-J Scholte (source: Knols & Thomas 2006).
1. *Metarhizium anisopliae*, sporulerend op een dood exemplaar van de Afrikaanse malariamug *Anopheles gambiae* s.s. De groenwitachtige kleur wordt veroorzaakt door miljoenen conidiën, die geproduceerd worden door conidiogene cellen, groeiend op de schimmelhyphen uit de dode mug.

control agents that opens up perspectives for vector control in Africa: fungi (Scholte *et al.* 2003a,b, 2004a,b, 2005, 2006, Blanford *et al.* 2005, Thomas *et al.* 2005, Knols & Thomas 2006).

Many entomopathogenic fungi have proven to be pathogenic to mosquitoes (Scholte *et al.* 2004b), and some of them have also been found in African malaria vectors (table 1). This list is likely to become much longer if fungal pathogens of the malaria vector *Anopheles gambiae* Giles sensu stricto are specifically being searched for in the field, and other fungi will be tested on this vector in the laboratory. While successful mosquito vector control in Africa is currently based on controlling adult mosquitoes, the vast majority of mosquitocidal fungi is aquatic and may only be used to control the aquatic stages of the insects. However, recent progress has been made in using anamorphic entomopathogenic fungi (Deuteromycetes) for controlling adult African mosquitoes (Scholte *et al.* 2003a,b, 2004a,b, 2005, Blanford *et al.* 2005). These fungi have a distinct advantage over the biological control agents mentioned above, in that they do not need to be ingested to infect and kill the insects – infection takes place through physical contact of the infective propagules (conidia) with the insect cuticle. As such, the contamination method is similar to conventional indoor residual spraying with insecticides. It is generally accepted that the use of these insect-pathogenic fungi, such as *Metarhizium anisopliae*, as a myco-insecticide is not harmful to the environment (Zimmermann 1993, Strasser *et al.* 2000), nor to humans. This was illustrated by the fact that in 2003, an *M. anisopliae* isolate (strain F52) was granted registration by the U.S. Environmental Protection Agency to be used against insect pests. The dossier stated that, after thorough (eco)toxicological testing, no harm is expected to be caused to humans or the environment (EPA 2003, Kanzok & Jacobs-Lorena 2006).

General life cycle of deuteromycetous entomopathogenic fungi

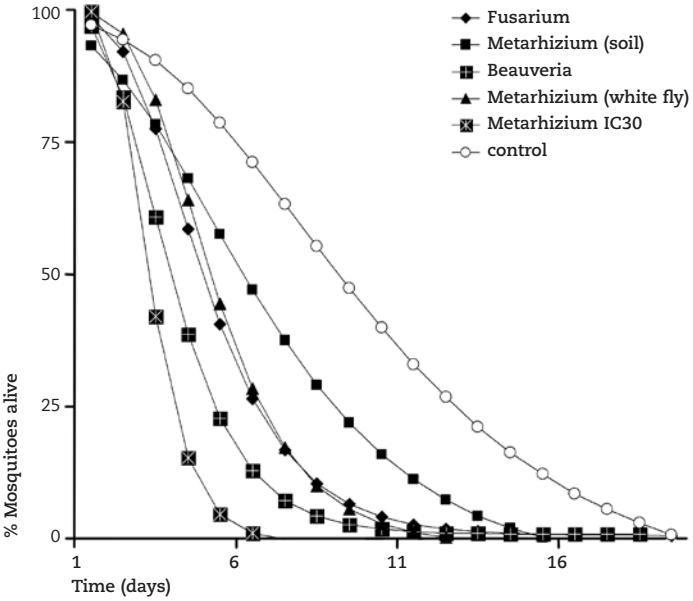
Deuteromycetous fungi are a group of fungi of which no sexual stage has been reported. Several insect pathogenic fungal genera such as *Beauveria*, *Fusarium* and *Metarhizium* belong to this group. The life cycle of entomopathogenic deuteromycetous fungi starts with conidia that attach to, germinate on, and penetrate the insect cuticle. Once in the haemocoel, the mycelium grows throughout the host, forming hyphal bodies called blastospores. Death of the insect is often due to a combination of the action of fungal toxins, physical obstruction of blood circulation, nutrient depletion and/or invasion of organs. After the host has died, hyphae usually emerge from the cadaver and, under suitable abiotic conditions, conidia are produced on the exterior of the host (figure 1). These are then dispersed by wind or water (Goettel & Inglis 1997).

First screening of candidate fungi

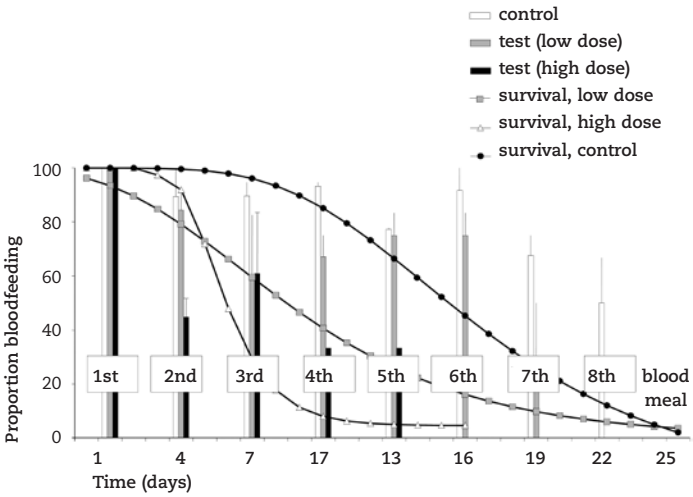
In order to obtain a suitable fungal candidate for controlling *An. gambiae* s.s., a search for mosquitocidal Deuteromycetes was conducted in Western Kenya (Scholte *et al.* 2003b). Four insect-pathogenic fungi were isolated. Three species were isolated from dead insects: *Beauveria bassiana* and *Fusarium* sp. from the stemborer *Busseola fusca* (Fuller), and an *M. anisopliae* strain from the white fly *Trialeurodes vaporariorum* (Westwood); one *M. anisopliae* isolate was found in a soil sample. A fifth fungus (*M. anisopliae* isolate ICIPE 30) was provided by the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya (courtesy N.K. Maniania). Unformulated conidia of these fungi were screened for their virulence against adult *An. gambiae* s.s. mosquitoes. Survival analysis (Kaplan Meier) showed that survival of mosquitoes that were infected with either one of the five fungi was significantly lower than that of the control group, although virulence varied between fungi (figure 2) (Scholte *et al.* 2003b). Mean infection percentages are depicted in table 2. From this initial fungal screening experiment, the hyphomycete *M. anisopliae* isolate ICIPE 30 proved to be the most virulent for *An. gambiae*, and subsequent experiments were carried out using this isolate.

Development of standard infection methodology

For contamination of adult mosquitoes with fungal conidia of a known concentration, a standard protocol was developed similar to screening of residual pesticides on mosquitoes: a filter paper was impregnated on one side with a known concentration of conidia, formulated with an 8% adjuvant vegetable oil suspended in 0.05% Tween-80 and dried slowly during 48 hrs at room temperature. The paper was rolled up and inserted into a



2. Survival curves (fitted on Gompertz survival distribution) of adult mosquitoes (*Anopheles gambiae* s.s.) infected with various entomopathogenic fungi (source: Scholte *et al.* 2003).
2. Overleving (geplot op de 'Gompertz-overlevingsverdeling') van volwassen *Anopheles gambiae* s.s., geïnfecteerd met verschillende soorten entomopathogene schimmels.



3. Survival curves (fitted on Gompertz distribution), from the second experiment on blood-feeding propensity, and proportions (± SE) of *M. anisopliae*-infected female *An. gambiae* s.s. that took blood meals, as calculated from the mosquitoes alive at the time when each successive blood meal was due (source: Scholte *et al.* 2006).
3. Overleving (geplot op de 'Gompertz-overlevingsverdeling'), uit het tweede experiment dat de neiging tot bloedvoeden onderzocht, en de relatieve aantallen (± SE) van *M. anisopliae*-geïnfecteerde vrouwelijke *An. gambiae* s.s. die een bloedmaaltijd namen, berekend op basis van de aantallen muggen die een volgende bloedmaaltijd namen.

Table 2. Infectivity and pathogenicity of various fungal species to adult *Anopheles gambiae* s.s. mosquitoes (source: Scholte *et al.* 2003).
Table 2. Mate van besmettelijkheid en ziekteverwekkend vermogen van enkele schimmelsoorten tegen volwassen stadia van *Anopheles gambiae* s.s. muggen.

Fungus	% Infected ± SE[1]	LT_{50} ± SE (days)	Grouping[2] LT_{50}
control		8.80 ± 0.66	A
M. anisopliae (soil-sample)	46.5 ± 3.6	5.87 ± 0.77	B
Fusarium sp.	79.0 ± 4.3	4.48 ± 0.21	B C
B. bassiana	81.7 ± 0.9	3.49 ± 0.29	C
M. anisopliae (white fly)	83.0 ± 3.4	4.71 ± 0.55	B C
M. anisopliae (ICIPE 30)	88.7 ± 3.3	3.39 ± 0.37	C

[1]SE: Standard error of the mean, [2]LT_{50} values without letters in common are significant at $p<0.05$

glass cylinder in such a way that the side containing conidia was the surface on which mosquitoes would rest once inserted in the glass cylinder. Subsequent laboratory experiments on female *An. gambiae* s.s. showed that a dose of 1.6 x 10^{10} conidia/m^2 resulted in infection percentages between 83.7 and 97.7, and LT_{50}-values (i.e., the time it takes for 50% of a group to die) ranging from 3.2 to 5.9 days, while uninfected females lived up to 31 days with LT_{50}s between 9.9 and 18.5 days (Scholte *et al.* 2003a, 2004a). This dosage was considered suitable for field application, because the expected infection proportions in targeted mosquitoes were high, and the quantities of required conidia were of an acceptable magnitude.

Fungal infection effects on blood-feeding and fecundity of mosquitoes

In addition to causing significant mortality, *M. anisopliae* is known to cause reductions in feeding and fecundity in a range of insects. Since a change in blood-feeding propensity of malaria vectors could have important implications regarding malaria transmission, we investigated whether blood feeding of *An. gambiae* s.s. was also affected by infection with *M. anisopliae*

Gonotrophic cycle	Treatment Control	Low fungal dosage	High fungal dosage
1	50.97 ± 4.17 (34)	50.13 ± 2.72 (56)	70.31 ± 4.79 (29)
2	65.09 ± 5.80 (32)	64.31 ± 5.56 (39)	36.65 ± 10.1 (23)
3	71.53 ± 7.27 (32)	58.29 ± 7.13 (28)	31.69 ± 9.89 (16)
4	80.96 ± 6.90 (27)	58.22 ± 8.93 (23)	24.80 ± 18.3 (5)
5	6.33 ± 9.87 (18)	44.91 ± 14.3 (11)	40.00 ± 13.3 (2)
6	59.23 ± 14.2 (13)	54.89 ± 15.6 (9)	0 (2)
7	51.67 ± 22.6 (3)	19.60 ± 6.53 (5)	0 (2)
8	0	0	0
average*	65.55 ± 2.96	54.81 ± 2.67	45.48 ± 4.70

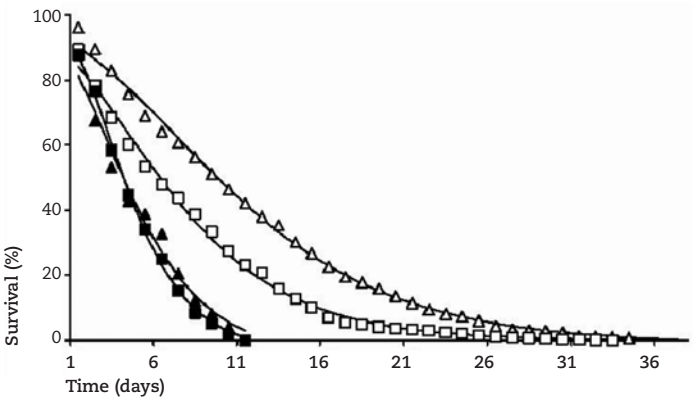
*significant overall effect of fungal infection on average number of eggs per bloodmeal (ANOVA): $F=9.784$; $p<0.001$, $df=2$.

Table 3. Mean number of eggs (± SE) laid per blood meal, as calculated from the female mosquitoes alive at the time when oviposition was due in each gonotrophic cycle (n) (source: Scholte *et al.* 2006).
Table 3. Gemiddelde aantal eitjes (± SE) dat gelegd werd per bloedmaaltijd, berekend op grond van het aantal vrouwtjesmuggen dat levend was op het moment waarop ze, per gonotrofische cyclus, verwacht werden eitjes te leggen (n).

4. (a): Position of black cloth (dotted line) impregnated with conidia of the entomopathogenic fungus *Metarhizium anisopliae* inside a Tanzanian house. (b): Blood-fed *An. gambiae* females on a *M. anisopliae*-infected cloth (source: Scholte *et al.* 2005).
4. (a): De positie van de met de entomopathogene schimmel geïmpregneerde zwarte doek (gestreepte lijn) in een Tanzaniaans huis. (b): Volgezogen *An. gambiae* vrouwtjes op een *M. anisopliae*-geïmpregneerd doek.

(Scholte *et al.* 2006). The mosquitoes were contaminated with either a low (1.6 x 10^8 conidia/m^2) or a moderately high (1.6 x 10^9 conidia/m^2) dose of oil-formulated *M. anisopliae* conidia, and offered a single human blood meal 48, 72, or 96 hrs later to assess feeding propensity and individual blood meal size. Females infected with the low dose of conidia ingested less blood than uninfected females [3.79 ± 1.34 µg *vs.* 7.78 ± 0.99 µg hematin/blood meal for large females (wing size ≥ 3.1 mm) and 2.88 ± 1.11 µg *vs.* 3.90 ± 0.82 µg hematin for small females (wing size < 3.1 mm)]. This experiment also showed that females took significantly fewer blood meals than uninfected females 72 hrs after they had been contaminated.

In a second experiment, individually kept fungus-infected females were offered a blood meal every third day (to a total of eight gonotrophic cycles), and allowed to oviposit after each cycle in order to quantify feeding propensity and fecundity. After each feeding opportunity it was scored whether the female had fed or not. It was found that mosquitoes, inoculated with the moderately high dose of conidia, exhibited reduced appetite (figure 3). Of the fungus-infected females, the proportion of mosquitoes taking a second blood meal was reduced with 51%. This was further reduced to 35.3% for the fourth blood meal. During eight feeding opportunities, the average number of blood meals taken by uninfected females was 4.39, against 3.40 (low dose) and 2.07 (high dose) blood meals for the fungus-infected females. Moreover, infected females produced fewer eggs per gonotrophic cycle (table 3).



5. Survival of uninfected (open symbols: △ = females, □ = males) and *M. anisopliae*-infected (closed symbols: ▲ = females, ■ = males) wild *An. gambiae* s.l. mosquitoes collected from rural Tanzanian houses (source: Scholte *et al.* 2005).
5. Overleving van ongeïnfecteerde (open symbolen: △ = vrouwtjes, □ = mannetjes) en *M. anisopliae*-geïnfecteerde (gesloten symbolen: ▲ = vrouwtjes, ■ = mannetjes) wilde *An. gambiae* s.l. muggen, verzameld van huisjes in een Tanzaniaans dorp.

Application in the field – can entomopathogenic fungi reduce malaria transmission?

Fundamental to the design of malaria control programmes is a basic understanding of the relationship between malaria transmission by mosquito vector populations and malaria prevalence, incidence, morbidity and mortality. One parameter that describes the intensity of malaria transmission is the Entomological Inoculation Rate (EIR), which is the rate at which sporozoite-positive bites are received. This function expresses the importance of mosquito survival and the number of blood meals taken, reductions of which have high impact on the intensity of malaria transmission (Charlwood 1997, Charlwood *et al.* 1997, Killeen *et al.* 2000, Drakeley *et al.* 2003). In a small-scale field study in Tanzania, we placed *M. anisopliae*-impregnated cloths indoors of local houses (figure 4) (Scholte *et al.* 2005). Of all *An. gambiae* sensu lato collected in these houses, 23% were found to be infected with the fungus. The infected mosquitoes had significantly shorter life spans compared to those of non-infected ones (figure 5). LT_{50}-values were 3.70 and 3.49 days for infected males and females, respectively, and 5.88 and 9.30 days for uninfected males and females. The reductions in daily survival rates of wild *An. gambiae* s.l. and numbers of blood meals taken by *M. anisopliae*-infected females may thus have a high impact on the EIR, provided that enough mosquitoes become infected. Extensive field studies on village scale have recently begun to assess the impact of this fungus on EIR and morbidity.

Important aspects concern the system of delivering the fungus to the mosquito population, and effective coverage of the

targeted insect population with the fungus. In the field study in Tanzania, conidia were suspended in an 8% adjuvant oil formulation and applied on 3-m² black cotton sheets. These sheets were placed inside houses on the ceiling and contaminated indoor-resting mosquitoes with the fungus. Increased size of these 'resting targets' and application in larger parts of human habitations may maximise coverage.

Application strategies

Killeen *et al*. (2004) showed that the EIR can be drastically reduced with methods that do not require chemicals but are based on integration of a few moderately efficient vector control methods such as environmental management-based larval control and zooprophylaxis. In a similar way we envisage an integrated vector-control programme without any use of chemicals, where *M. anisopliae* is used against adult mosquito vectors in addition to biological larval control with *Bacillus thuringiensis* subsp. *israelensis* and/or *B. sphaericus* (Fillinger *et al*. 2003), and application of untreated bed nets, push-pull methods with zooprophylaxis (Seyoum *et al*. 2002) and repellent plants (Seyoum *et al*. 2003). Larval control will reduce the number of emerging adults, whereas cattle in or near houses may prevent some of the vectors from feeding on humans. Repellent odours from live, potted, or burned plants around houses will diminish the numbers of mosquitoes entering the houses. Mosquitoes which are able to enter dwellings will be prevented to bite by bed nets (Guyatt & Snow 2002, Takken 2002). And if some of these mosquitoes that entered houses will be infected by *M. anisopliae* when they land on the 'resting targets', this will reduce their survival rate. None of these techniques, as stand-alone tools, is perfect, but the combination of these vector control methods may be very powerful and greatly enhance reductions of the EIR. Such a strategy may result in a more sustainable vector-borne disease control than the current ones based on potentially harmful chemicals. However, in order to be successful, substantial financial, political and local support for long-term and large-scale application is necessary.

References

Becker N & Ascher KRS 1998. The use of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) against mosquitoes, with special emphasis on the ecological impact. Israel Journal of Entomology 32: 63-69.

Becker N, Zgomba M, Petric D, Dahl C, Boase C, Lane J & Kaiser A 2003. Mosquitoes and their control. Kluwer Academic / Plenum Publishers.

Blanford S, Chan BHK, Jenkins N, Sim D, Turner RJ, Read AF & Thomas MB 2005. Fungal pathogen reduces potential for malaria transmission. Science 308: 1638-1641.

Brooke BD, Hunt RH, Chandre F, Carnevale P & Coetzee M 2002. Stable chromosomal inversion polymorphisms and insecticide resistance in the malaria vector mosquito *Anopheles gambiae* (Diptera: Culicidae). Journal of Medical Entomology 39: 568-573.

Chandre F, Darriet F, Manga L, Akogbeto M, Faye O, Mouchet J & Guillet P 1999. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bulletin World Health Organ 77: 230-234.

Chapman HC 1974. Biological control of mosquito larvae. Annual Review of Entomology 19: 33-59.

Charlwood JD 1997. Vectorial capacity, species diversity and population cycles of anopheline mosquitoes (Diptera: Culicidae) from indoor light-trap collections in a house in southeastern Tanzania. African Entomology 5: 93-101.

Charlwood JD, Smith T, Billingsley PF, Takken W, Lyimo EOK & Meuwissen JHET 1997. Survival and infection probabilities of anthropophagic anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. Bulletin of Entomological Research 87: 455-453.

Drakeley C, Schellenberg D, Kihonda J, Sousa CA, Arez AP, Lopez D, Lines J, Mshinda H, Lengeler C, Armstrong-Schellenberg J, Tanner M & Alonso P 2003. An estimation of the entomological inoculation rate for Ifakara: a semi-urban area in a region of intense malaria transmission in Tanzania. Tropical Medicine & International Health 8: 767-774.

Fillinger U, Knols BGJ & Becker N 2003. Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. Tropical Medicine & International Health 8: 37-47.

Goettel MS & Inglis GD 1997. Fungi: Hyphomycetes. In: Lacey LA (ed) Manual of techniques in insect pathology: 213-249. Academic Press.

Guyatt HL & Snow RW 2002. The cost of not treating bednets. Trends in Parasitology 18: 12-16.

Hemingway J & Ranson H 2000. Insecticide resistance in insect vectors of human disease. Annual Review of Entomology 45: 371-391.

Kanzok SM & Jacobs-Lorena M 2006. Entomopathogenic fungi as biological insecticides to control malaria. Trends in Parasitology 22: 49-51.

Kaya HK & Gaugler R 1993. Entomopathogenic nematodes. Annual Review of Entomology 38: 181-206.

Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF & Beier JC 2000. The potential impact of integrated malaria transmission control of entomologic inoculation rate in highly endemic areas. American Journal of Tropical Medicine and Hygiene 62: 545-551.

Killeen GF, Seyoum A & Knols BGJ 2004. Rationalizing historical success of malaria control in Africa in terms of mosquito resource availability management. American Journal of Tropical Medicine and Hygiene 71 (suppl): 87-93.

Knols BGJ & Thomas M 2006. Fungal entomopathogens for adult mosquito control – a look at the perspectives. Outlooks on Pest Management 17: 1-3.

Legner EF 1995. Biological control of Diptera of medical and veterinary importance. Journal of Vector Ecology 20: 59-120.

Roberts DW 1967. Some effects of *Metarhizium anisopliae* and its toxins on mosquito larvae. In: Laan PA van der (ed) Insect pathology and microbial control: 243-246. North-Holland Publishing Company.

Scholte EJ 2004a. The entomopathogenic fungus *Metarhizium anisopliae* for mosquito control. Impact on the adult stage of the African malaria vector *Anopheles gambiae* and filariasis vector *Culex quinquefasciatus*. PhD-thesis, Wageningen University.

Scholte EJ, Knols BGJ, Samson RA & Takken W 2004b. Entomopathogenic fungi for mosquito control: A review. Journal of Insect Science 4: 19-42.

Scholte EJ, Knols BGJ & Takken W 2006. Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces blood-feeding and fecundity. Journal of Invertebrate Pathology 91: 43-49.

Scholte EJ, Ng'habi K, Kihonda J, Takken W, Paaijmans K, Abdulla S, Killeen GF & Knols BGJ 2005. An entomopathogenic fungus for control of adult African malaria mosquitoes. Science 308: 1641-1642.

Scholte EJ, Njiru BN, Smallegange RC, Takken W & Knols BGJ 2003a. Infection of malaria (*Anopheles gambiae* s.s.) and filariasis (*Culex quinquefasciatus*) vectors with the entomopathogenic fungus *Metarhizium anisopliae*. Malaria Journal 2: 29-36.

Scholte EJ, Takken W & Knols BGJ 2003b. Pathogenicity of five East African entomopathogenic fungi to adult *Anopheles gambiae* s.s. (Diptera: Culicidae) mosquitoes. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV, Amsterdam) 14: 25-29.

Seyoum A, Balcha F, Balkew M, Ali A & Gebre-Michael T 2002. Impact of cattle keeping on human biting rate of anopheline mosquitoes and malaria transmission around Ziway, Ethiopia. East African Medical Journal 79: 485-489.

Seyoum A, Killeen GF, Kabiru EW, Knols BGJ & Hassanali A 2003. Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. Tropical Medicine & International Health 8: 1005-1011.

Strasser H, Vey A & Butt TM 2000. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? Biocontrol Science and Technology 10: 717-735.

Takken W 2002. Do insecticide-treated bednets have an effect on malaria vectors? Tropical Medicine & International Health 7: 1022-1030.

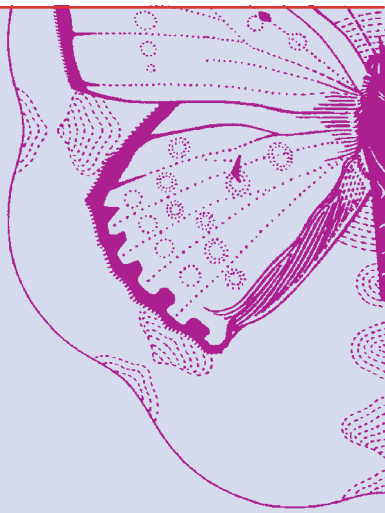
Thomas MB, Blanford S, Jenkins NE, Killeen GF, Knols BGJ, Read AF, Scholte E-J & Takken W 2005. Benefits and risks in malaria control: A response. Science 310: 49.

Zimmermann G 1993. The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. Pesticide Science 37: 375-379.

Ontvangen: 26 maart 2007
Geaccepteerd 16 december 2007

Samenvatting

Een insectpathogene schimmel (*Metarhizium anisopliae*) ter bestrijding van volwassen Afrikaanse malariamuggen (*Anopheles gambia*)
Bestrijding van malariamuggen in Afrika gebeurt vrijwel geheel met chemische insecticiden. Hoewel er diverse biologische bestrijdingsorganismen bestaan die larvale populaties kunnen decimeren, zijn er geen organismen die kunnen worden ingezet voor de bestrijding van het volwassen stadium. Dit artikel beschrijft een serie laboratorium- en veldexperimenten waarin een nieuwe biologische bestrijdingsmethode wordt geëvalueerd. Deze methode gebruikt een insect-pathogene schimmel (*Metarhizium anisopliae*) die wordt ingezet tegen adulten van de Afrikaanse malariamug *Anopheles gambiae* sensu stricto. Diverse aspecten van deze nieuwe methode worden besproken, waaronder de beoogde toepassingstechniek, sublethale effecten van de schimmel op een geïnfecteerde mug, de impact die gebruik van deze methode kan hebben op malariaoverdracht, en hoe deze nieuwe methode deel kan uitmaken van een geïntegreerde benadering van muggenbestrijding in de tropen.



Ernst-Jan Scholte
Dutch Plant Protection Service
P.O. Box 9102
6700 HC Wageningen
The Netherlands
e.j.scholte@minlnv.nl

Bart G.J. Knols, Willem Takken
Laboratory of Entomology
Wageningen University and Research Centre
P.O. Box 8031
6700 EH Wageningen
The Netherlands