Tropical Medicine and International Health

VOLUME 8 NO 1 PP 37–47 JANUARY 2003

Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya

Ulrike Fillinger[1,3], Bart G. J. Knols[1,2] and Norbert Becker[3]

1 *International Centre of Insect Physiology and Ecology (ICIPE), Mbita Point, Kenya*
2 *Laboratory of Entomology, Wageningen University Research Centre, Wageningen, The Netherlands*
3 *German Mosquito Control Association (KABS), Waldsee, Germany*

Summary

We evaluated the efficacy of new water-dispersible granular (WDG) formulations of *Bacillus thuringienis* var. *israelensis* (*Bti*; VectoBac®) and *B. sphaericus* (*Bs*; VectoLex®, Valent BioScience Corp., Illinois, USA) for the control of larval *Anopheles gambiae sensu lato* Giles mosquitoes in a malaria-endemic area around Lake Victoria, Western Kenya. WDG and powder formulations were compared in laboratory bioassays and followed by efficiency and residual effect assessments of both WDG formulations in open field experiments. LC_{50} and LC_{95} values for the *Bti/Bs* strains and their formulations show high susceptibility of *A. gambiae sensu stricto* under laboratory conditions. The larvae proved more susceptible to *Bs* than to *Bti* and the WDG formulations were slightly superior to the powder formulations. High efficiency was also shown in the open field trials, and a minimum dosage of 200 g/ha *Bti* WDG, representing the LC_{95} of the laboratory tests, was sufficient to fully suppress emergence of mosquitoes when applied at weekly intervals. *Bti* WDG did not show a residual effect, irrespective of the concentration applied. The *Bs* WDG formulation, however, showed significant larval reductions up to 11 days post-treatment at application doses of either 1 or 5 kg/ha. We conclude that the main malaria vector in our study area is highly susceptible to these microbial control agents. Minimum effective dosages to achieve elimination of the larval population in a given habitat are extremely low and environmental impact is negligible. Microbial products for larval control have therefore great potential within Integrated Vector Management programmes and may augment control efforts against adult vector stages, such as the use of insecticide-treated bednets, in many parts of Africa.

keywords *Anopheles gambiae*, *Bacillus thuringienis* var. *israelensis*, *Bacillus sphaericus*, water-dispersible granule, malaria vector, microbial, larvicide, mosquito control, Kenya

correspondence Dr U. Fillinger, ICIPE, Mbita Point Research and Training Centre, PO Box 30, Mbita, Kenya. Tel.: +254 385 22210/13/16; Fax: +254 385 22190; E-mail: ufillinger@mbita.mimcom.net

Introduction

Recent attempts to quantify the intolerable burden of malaria in Africa (Breman 2001) confirm that the true impact of the disease may have been underestimated for quite a long time. Hence, whilst faced with tremendous levels of drug resistance (Trape 2001), increasing insecticide resistance (Chandre *et al.* 1999; Hargreaves *et al.* 2000) and meagre resources at national levels, it is becoming evident that the ambitious goals of the WHO Roll Back Malaria campaign (Nabarro & Tayler 1998) now require an exceptionally high effort from governments and the research/control community alike in order to be successful. Besides early diagnosis and prompt treatment with effective drugs, it is clear that any possible means of reducing man–vector contact should be employed, either through vertical, but preferably through horizontally (participatory) staged programmes (Rozendaal 1997).

Vector control in Africa can target all stages of the mosquito life cycle, but has historically focused almost exclusively on adult control based on indoor residual house spraying (Mnzava *et al.* 1993; Curtis 1994; Roberts *et al.* 2000) or, more recently, the use of insecticide treated bednets or curtains (Lengeler 2001). The convincing

impact of such tools on childhood mortality and morbidity has been extremely useful not only because of the lives directly protected but also because it has restored confidence in vector control as a valid prevention tool in Africa. However, the significant success of bednets has also diverted interest from other strategies, including historically successful but much neglected approaches such as larval control. Truly integrated and well-managed efforts, as staged during the early decades of the last century in Zambia (Utzinger *et al.* 2001), yielded exceptionally high successes and incorporated environmental management, bednets and curative drugs. Major successes of larval control operations against African vector species, e.g. the eradication of *Anopheles gambiae* from Brazil (Soper & Wilson 1943) and Egypt (Shousha 1948) in the pre-dichlorodiphenyltrichloroethane era, have largely been forgotten (Killeen *et al.* 2002). At the same time, malaria was successfully eradicated in the United States, Europe and the Middle East by rigorous larval control measures, integrated with any other available tool (Kitron & Spielman 1989; Hays 2000). The national malaria eradication programmes that followed succeeded because of the application of both old and newly developed techniques (Hays 2000).

Larviciding and source reduction have a major advantage in that they control mosquitoes before they disperse and transmit disease (Killeen *et al.* 2002). Soper and Wilson (1943) were extremely successful in eradicating *A. gambiae* from north-east Brazil from large areas of ideal habitat where the vector proliferated and became infected with parasites at rates that far outweighed most African settings. But they deployed highly toxic agents such as arsenic-based Paris green and petroleum oils, affecting the environment and field workers alike. With the discovery of the mosquitocidal strains of *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*) and *B. sphaericus* Neide (*Bs*) during the mid-1970s, larvicides have become available that are highly effective, yet selective in action (Charles & Nielsen-LeRoux 2000), and therefore environmentally safe to non-target organisms (WHO 1999), as well as for human exposure (WHO 1999). These *Bacillus* products are characterized by the ease of handling, cost-effectiveness and capability of being produced locally. Furthermore, application of larvicides does not require expensive equipment, can be organized locally (Becker 1992) and is, according to our experience thus far, highly acceptable in the community. The great advantage of *Bti* over all other larvicides is the low probability of developing resistance (Charles & Nielsen-LeRoux 2000).

Considerable research has been conducted on bacterial insecticides over the last decades, and major successes have been obtained. The significant advantages of *Bti* and *Bs* over chemical insecticides have been responsible for their fast introduction into large-scale routine operations for mosquito control in Europe (Becker 1998) as well as for blackfly (Simuliidae) control in Africa (Guillet *et al.* 1990), within a mere 5 years of their discovery. Much of the success of the West African onchocerciasis control programme has been attributed to the availability of *Bti* as an alternative to synthetic chemical insecticides and their associated resistance and environmental problems.

An increasing number of countries in the tropics are realizing the need to integrate microbial larvicides into their mosquito control programmes. Especially in South America, efforts are underway to test new, local strains and formulations (Consoli *et al.* 1997; Rodrigues *et al.* 1998, 1999) and to integrate them with strategies against adult vectors (Kroeger *et al.* 1995a,b; Blanco Castro *et al.* 2000; Regis *et al.* 2000a,b). Most of these developments are driven by insecticide resistance, environmental hazard and the recognition of old tools, proven to be useful in the past.

Relatively few studies with *Bti/Bs* have been carried out against African malaria vectors (Majori *et al.* 1987; Karch *et al.* 1991, 1992; Ragoonanansingh *et al.* 1992; Ravoahangimalala *et al.* 1994; Seyoum & Abate 1997; Skovmand & Sanogo 1999) and were restricted to experimental and operational research (Barbazan *et al.* 1997, 1998), but not large-scale control operations. Nevertheless, these studies have underlined the potential of larval control using microbial insecticides and deserve broader dissemination and application.

The development of new products, easy to handle in the field, suitable for long distance transport, storage under tropical climate conditions and high efficacy against mosquito vectors of disease is a prerequisite for these larvicides to enter the vector control arena. Our study is the first to evaluate water-dispersible granule (WDG) formulations against malaria mosquitoes in Africa in preparation for upcoming large-scale trials in Western Kenya.

Materials and methods

Study area

The research was carried out at the ICIPE Mbita Point Research and Training Centre, in Suba District, located on the shores of Lake Victoria, Nyanza Province, Western Kenya (altitude 1100–1300 m). The Luo tribe, practising fishing and subsistence farming, densely populates the study area. Malaria in the district is representative of endemicity levels encountered throughout the lake region, and is the main cause of childhood mortality and hospital admission (Mutero *et al.* 1998). The two rainy seasons (average annual rainfall 1150 mm) from March to June

and October to November are not very well defined; some years being characterized by more or less continuous rains and others by prolonged dry periods. Malaria transmission fluctuates seasonally but is sustained all year round by the three malaria vectors *A. gambiae* Giles, *A. arabiensis* Patton and, to a lesser extent, *A. funestus* Giles. They breed in a variety of natural and man-made larval habitats. In the township of Mbita Point, *A. gambiae sensu stricto* is the predominant species among adults resting in houses (Minakawa *et al.* 1999), and breeding in man-made habitats (e.g. burrow pits) is common.

Mosquitoes

All laboratory experiments were carried out with third instar larvae of laboratory-reared *A. gambiae s.s.*, which were originally colonized from specimens collected at Njage village, 70 km from Ifakara, South-East Tanzania, in April 1996. Larvae were reared in round plastic tubs (diameter 0.6 m) filled with water from Lake Victoria and fed Tetramin® (Pfitzer Inc., UK) fish food twice daily. Experimental larvae were randomly collected from several tubs to compensate for size differences and feeding history which are known to be influenced by larval density (Lyimo & Takken 1993).

The open field trials were conducted with offspring of wild *A. gambiae sensu lato* females that oviposited in the experimental tub (see below). Colonization of experimental tubs occurred within 2 days, and sometimes included larvae of *Culex quinquefasciatus* Say and *C. tigripes* Grandpre & Charmoy.

Bacillus formulations

Test strains of *Bti* and *Bs* were kindly provided by Valent BioSciences Corp., IL, USA and included two WDG formulations and two technical powders. The *Bti* formulations were either VectoBac® (Valent BioSciences Corp, Illinois, USA) WDG formulation (designated as ABG-6511, lot 47-068-BR; potency 2700 ITU [International Toxic Units]/mg], or Bactimos® (Valent BioSciences Corp, Illinois, USA) primary powder (PP) (lot 31-526-PG; potency 10 000 ITU/mg). Potencies were determined in reference to the standard ISP 82. The *Bs* formulations were either VectoLex® WDG formulation (lot 56-809-PG; potency 650 BsITU/mg; reference according to manufacturer), or technical powder (TP) (lot BSB 0004; potency of 1600 BsITU/mg; in reference to standard SPH 88).

Whereas the WDG formulations dispersed readily when mixed with water and remained like that for at least several minutes, it was noted that the technical powders needed to be mixed thoroughly. In spite of this it was observed that the powders settled rapidly on the bottom of the vials, unlike the formulated WDG products.

Laboratory assays

Prior to open field evaluations, the WDG formulations of *Bti* and *Bs* were tested in the laboratory to determine the minimum effective dosages, according to standard testing procedures (WHO 1996). For comparison, technical powders were evaluated simultaneously. For each formulation (four in total), preliminary tests were conducted in three replicates of three to six different concentrations, and controls (water from Lake Victoria only) in order to determine their respective active ranges. Test concentrations were obtained through sequential dilution of a stock solution (100 mg of product in 1 l distilled water).

Following theses preliminary trials, the needed test aliquots were added to 1 l of lake water dispensed in plastic 1.5 l containers, which contained 50 larvae each. Larvae were not fed during the experiments and mortality was scored after 24 h. Moribund larvae were considered dead and included in the analyses. If mortality in the control treatment exceeded 10% the test was discarded and repeated. The bioassays where run in five to six different concentrations (ranging from 0.01 to 2 p.p.m.) plus controls and replicated thrice, on two different occasions. All trials were conducted at ambient temperatures that ranged from 23 to 30 °C. Data from all replicates were pooled and analysed using computer software (Reymond 1985) for probit-regression analysis after Finney (1971).

Open field trials

Open field trials with the WDG formulations were conducted between February–April 2000 (dry season) and April–May 2001 (rainy season). Eighteen plastic tubs (diameter 0.6 m) were buried into an open sunlit field without vegetation in two lines (1.5 m apart) of nine tubs (distance between tubs 1.5 m). Soil and mud from known *A. gambiae* breeding sites was added to each tub (one-third of its volume) to provide suitable biotic and abiotic conditions for mosquitoes. Tubs were subsequently filled with water from Lake Victoria to a depth of 0.2–0.3 m. After colonization of the tubs, completion of the larval life cycle was found to take only up to 10 days, due to high water temperatures (25 ± 2 °C in the morning, 31 ± 2 °C in mid-afternoon). In order to ensure adequate numbers of third and fourth instar larvae, treatments were not undertaken before 8 days after setting the tubs. Care was taken not to allow adult mosquitoes to emerge, by removing mature pupae from all tubs twice a day.

Treatment concentrations were calculated on the basis of a standard water depth of 0.1 m and fixed surface area (Schnetter *et al.* 1981; Ragoonanansingh *et al.* 1992), and ranged from 0.2 to 1.6 mg/l (equals surface application of 0.2–1.6 kg/ha) for the *Bti* tests and from 1 to 5 mg/l (1–5 kg/ha) in the *Bs* tests. During each replicate, six tubs served as controls, whereas each half of the remaining 12 tubs would receive a given concentration of the test formulation. Tubs were matched on the basis of larval density so that control and test treatment tubs had similar densities at the start of the experiment. *Bti*/*Bs* concentrations were selected on the basis of laboratory results and studies reported elsewhere (Karch *et al.* 1992; Romi *et al.* 1993; Barbazan *et al.* 1997, 1998; Tianyun & Mulla 1999).

Considering that the laboratory tests are conducted under standardized conditions without major abiotic and biotic influences, the LC values represent only minimum dosages. Under field conditions application rates normally have to be increased up to several times the LC_{95} to obtain sufficient larval control (Becker & Rettich 1994). *Bti* WDG was not considered to show a long residual effect. Therefore, the search for the optimum effective dosage is the search for the minimum dosage required for 100% larval mortality 48 h after application. In case of *Bs* treatments, the optimum dosage is considered to show a higher residual effect, known to be enhanced by high dosages (Pantuwatana *et al.* 1989). The tested *Bti* WDG concentrations represent one, two, four and eight times its LC_{95}, the tested *Bs* WDG concentrations 25 and 50 times its LC_{95}. The formulations were applied with one hand-held sprayer with a fixed volume (250 ml) per tub and sprayed evenly over the entire water surface. Afterwards all tubs were examined daily and the average number of larvae calculated per standard 250 ml capacity mosquito dipper by taking five dips per tub, four from the periphery and one from the centre. Immature mosquitoes were classified in three categories: early instars (first and second), late instars (third and fouth) and pupae. All larvae were counted, classified to genus and development stage and then returned to their respective sites.

The percentage reduction in larval mosquito densities was calculated using the formula of Mulla *et al.* (1971) which takes into account that natural changes (for instance through predation) in the mosquito larval populations are taking place at the same level and rate in both treated and untreated sites:

$$\text{Percentage reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100$$

where C_1 and C_2 describe the average number of larvae in the control tubs pre- and post-treatment, like T_1 and T_2 for the tubs treated with experimental formulations. The average number of all larval instars, late instars, and pupae in the control and treatment tubs were compared daily by non-parametric Kruskal–Wallis one-way ANOVA on ranks ($\alpha = 0.05$) and Kruskal–Wallis multiple-comparison Z-value test ($\alpha = 0.05$). NCSS software (Hintze 1997) was used for all analyses.

Results

Laboratory assays

Laboratory bioassays with *Bti* (VectoBac® WDG, ABG-6511, 2700 ITU/mg) against third instar larvae of *A. gambiae s.s.* showed that after 24 h of exposure, average concentrations of 57 ITU/l (0.021 mg/l) and 567 ITU/l (0.21 mg/l) caused 50 and 95% mortality, respectively (Table 1). Tests with the *Bs* product (VectoLex® WDG, 650 BsITU/mg) yielded similar mortalities at concentrations of 2.3 BsITU/l (0.04 mg/l) and 19.5 BsITU/l (0.038 mg/l). Values for the powder formulation of *Bti* (Bactimos® PP, lot 31-526-PG, 6500 ITU/mg) were slightly higher than those obtained with the WDG formulation at 60 (0.006 mg/l) and 585 ITU/l (0.09 mg/l) as the LC_{50} and LC_{95}, respectively. Similarly, the powder formulation of *Bs* (BSB 0004, 1600 BsITU/mg) showed slightly higher LC_{50} and LC_{95} values of 3.1 (0.002 mg/l) and 28.8 BsITU/l (0.018 mg/l), respectively. The above values represent the minimum effective dosages against *A. gambiae s.s.*

Table 1 Laboratory bioassay results of water-dispersible granular (WDG) and powder/technical formulations (PP/TP) of *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *B. sphaericus* (*Bs*) against third instar larvae of *Anopheles gambiae s.s.* after 24-h exposure

Formulation	LC_{50} (95% CL)*	LC_{95} (95% CL)	Slope ± SE	χ^2 (d.f.)
Bti WDG (2700 ITU/mg)	0.021 (0.012 < LC < 0.035)	0.210 (0.129 < LC < 0.468)	1.659 ± 0.251	3.45 (3)
Bs WDG (650 BsITU/mg)	0.004 (0.003 < LC < 0.005)	0.038 (0.022 < LC < 0.096)	1.593 ± 0.226	0.24 (3)
Bti PP (10 000 ITU/mg)	0.006 (0.004 < LC < 0.009)	0.090 (0.006 < LC < 0.242)	1.391 ± 0.230	4.22 (3)
Bs TP (1600 BsITU/mg)	0.002 (0.001 < LC < 0.003)	0.018 (0.010 < LC < 0.047)	1.699 ± 0.260	2.20 (4)

* Concentrations in p.p.m., CL, confidence limits; SE, standard error; d.f., degrees of freedom.

Table 2 Effects of *Bacillus thuringiensis* var. *israelensis* (*Bti*) WDG formulations on densities of late and early instar *A. gambiae s.l.* and per cent reduction in open field conditions during three subsequent treatments (T) with varying concentrations

	Average number per dip									Percentage reduction					
	Total instars			Late instars			Early instars			Total instars		Late instars		Early instars	
Day	Control	T_1	T_2	Control	T_1	T_2	Control	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
0*	15.0	14.4	15.3	7.3	5.2	4.5	7.7	9.2	10.8	–	–	–	–	–	–
1	19.3	0.6	0	9.8	0.2	0	9.5	0.4	0	97	100	98	100	96	100
2	17.4	2.6	3.3	10.7	0.1	0	6.7	2.5	3.3	84	81	99	100	70	66
3	17.1	16.6	6.4	7.3	0	0	9.8	16.6	6.4	0	63	100	100	0	54
4	13.7	10.5	5.6	6.4	0.2	0	7.3	10.3	5.6	20	60	96	100	0	47
5	9.9	11.6	4.8	4.8	1.1	1.2	5.1	10.5	3.6	0	52	67	60	0	50
6	7.7	11.8	5.9	3.6	1.7	2.1	4.1	10.1	3.8	0	25	32	3	0	36
7*	4.9	11.6	6.0	1.4	3.8	2.1	3.5	7.8	3.9	0	0	0	0	0	21
8	2.6	0	0.2	1.0	0	0.2	1.6	0	0	100	94	100	88	99	98
9	3.3	0	0	0.8	0	0	2.5	0	0	100	100	100	100	100	99
10	6.0	3.1	1.9	0.7	0.1	0	5.3	3.0	1.9	79	74	97	100	77	68
11	8.2	9.0	3.3	0.6	0.2	0	7.6	8.8	3.3	56	67	88	96	54	62
12	6.0	3.3	1.4	0.3	0.6	0.5	5.7	2.7	0.9	78	81	30	0	81	86
13	7.5	6.3	5.9	1.6	1.0	0.7	5.9	5.3	5.2	66	35	77	70	64	22
14*	6.2	5.9	6.4	1.5	1.5	1.3	4.7	4.4	5.1	62	15	61	36	63	6
15	3.4	0.4	0.2	2.1	0.2	0.2	1.3	0.2	0	88	94	91	91	84	98
16	2.6	0.3	1.0	2.0	0	0.1	0.6	0.3	0.9	88	63	100	96	49	0
17	3.2	2.3	5.1	1.9	0	0	1.3	2.3	5.1	25	0	98	100	0	0
18	3.7	3.3	5.8	0.9	0.6	1.6	2.8	2.7	4.2	6	0	40	0	0	0
19	2.8	3.4	5.1	0.4	0.5	1.5	2.4	2.9	3.6	0	0	0	0	0	0
20	2.6	4.1	3.8	0.5	1.7	1.9	2.1	2.4	1.9	0	0	0	0	0	18
21	2.6	3.7	4.7	0.7	1.3	2.6	1.9	2.4	2.1	0	0	0	0	0	0

* Day of application.
Day 0: $T_1 = 0.8$ mg/l, $T_2 = 1.6$ mg/l; Day 7: $T_1 = 0.8$ mg/l, $T_2 = 0.4$ mg/l; Day 14: $T_1 = 0.2$ mg/l, $T_2 = 0.4$ mg/l.

Open field trials

The effect of *Bti* and *Bs* WDG formulations on both *Anopheles* and *Culex* larvae was determined and no significant difference in terms of larval mortality was found between the two genera. *Culex* provided only up to 15% of the total larval population in the trials and therefore results from both genera were pooled.

Prior to the application of *Bti* WDG both the control and treatment containers had statistically similar densities averaging 14–15 larvae per dip. The per cent reduction of larvae following *Bti* application is shown in Table 2. Three treatments took place at weekly intervals, when different concentrations were tested. The mean numbers of total instars, late instars and pupae in control and treatment sites are shown in Figure 1. *Bti* WDG provided 88–100% mortality within 24 h at all doses of application. Whereas the amount of larvae in the control during the first treatment period increased, it naturally declined thereafter which could have been caused by a reduction in oviposition attractancy often observed in ageing breeding sites and/or increase in predator density. Considering the late instars only, reduction rates of 88–100% could be observed up to the fourth day after treatment. Residual activity of the *Bti* formulation, however, was very low as dips taken 2–3 days after treatment indicated quick and continuing re-colonization of all treated sites by early instars.

All concentrations tested were equally effective up to 2 days post-treatment for the total number of larvae and up to 4 days when considering the late instars only. No significance could be shown between the different concentrations tested at any time. More importantly, pupation levels were very low in the treated ponds (Figure 1), which is considered the most important parameter for efficacy assessment of larval control measures (Tianyun & Mulla 1999). All treatments, irrespective of the concentration, were equally effective at lowering pupal populations and an overall reduction in mosquito emergence of 95% was achieved.

The effects of a single application of *Bs* WDG on larval densities and the percentage reduction in comparison with untreated containers is shown in Figure 2 and Table 3.

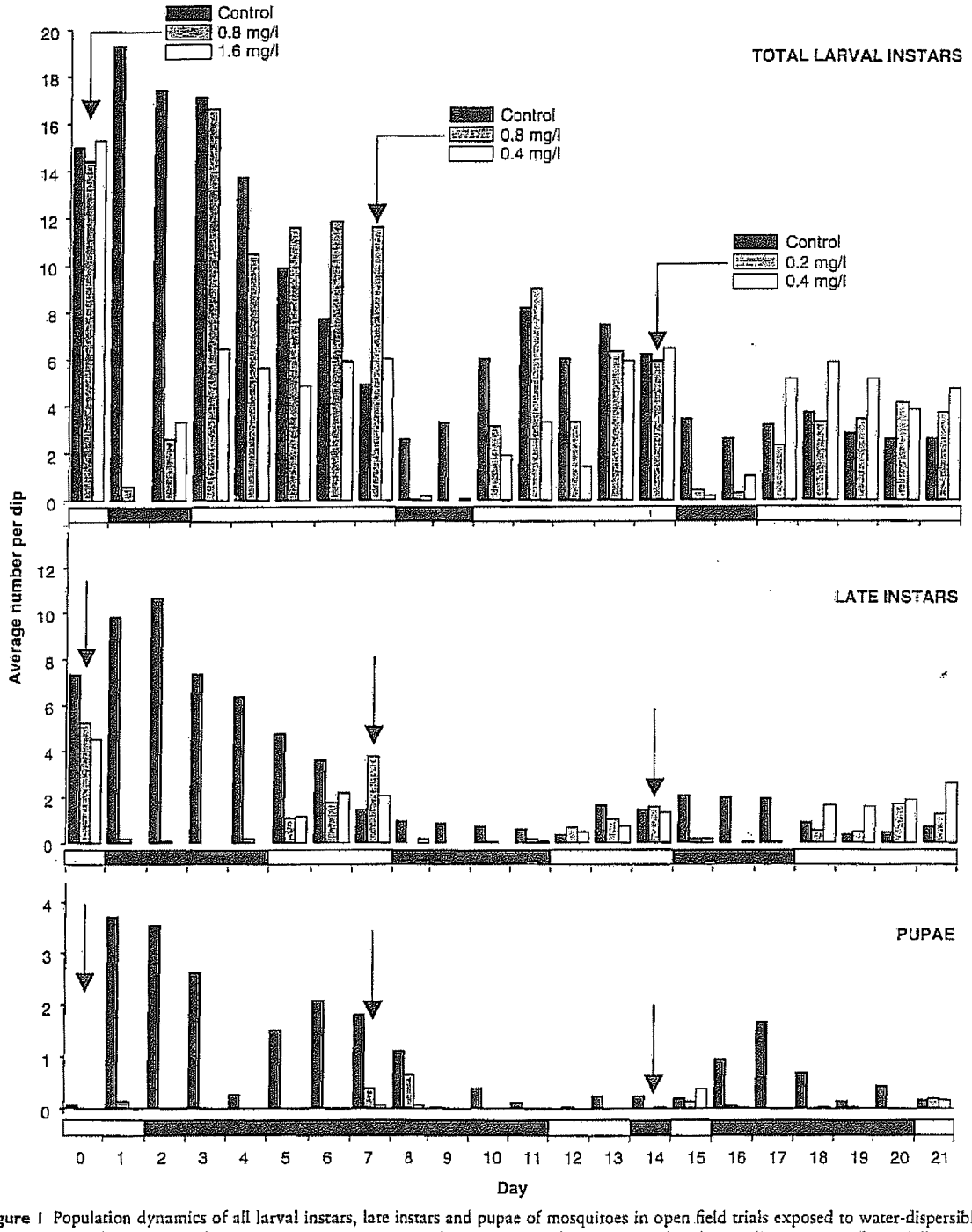

Figure 1 Population dynamics of all larval instars, late instars and pupae of mosquitoes in open field trials exposed to water-dispersible granule (WDG) formulation of *Bti* (2700 ITU/mg). Arrows indicate the date of treatment. White bars indicate no significant difference between treated and control tubs, black bars do ($\alpha = 0.05$).

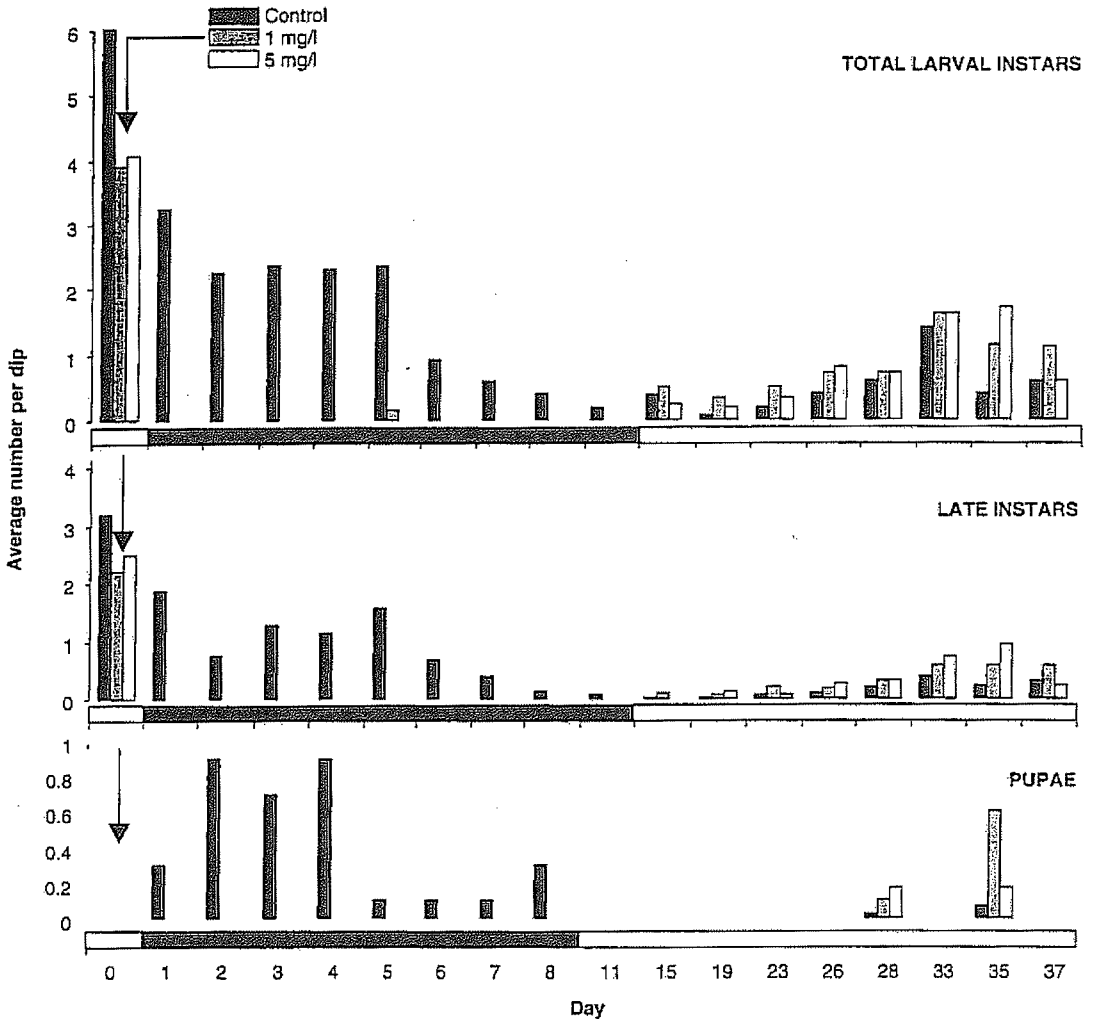

Figure 2 Population dynamics of all larval instars, late instars and pupae of mosquitoes in open field trials exposed to water-dispersible granule (WDG) formulation of *Bs* (650 BsITU/mg). Arrows indicate the date of treatment. White bars indicate no significant difference between treated and control tubs, black bars do ($\alpha = 0.05$).

The formulation provided 100% larval mortality within 24 h at both rates of application. In contrast with the *Bti* formulation, a prolonged residual effect, similar for both concentrations and lasting up to 11 days, was observed. Not a single larva survived during this period. Thereafter, however, densities in the control tubs dropped to low levels for reasons mentioned above, which made comparisons invalid. Overall, complete inhibition of emergence of adult mosquitoes was observed up to 2 weeks post-treatment.

Discussion

Microbial larvicides have several advantages over other mosquito control agents: not only high efficacy but also environmental safety and safety for human consumption, for instance when applied in drinking water (WHO 1999), makes them powerful vector control tools that are gaining more ground for disease control in Africa and other parts of the tropics. It is imperative therefore that new formulations, which are suitable for use in tropical

Table 3 Effects of *Bacillus sphaericus* (*Bs*) WDG formulations on densities of late and early instar *A. gambiae s.l.* and per cent reduction in open field conditions after a single application at two doses

	Average number per dip									Percentage reduction					
	Total instars			Late instars			Early instars			Total instars		Late instars		Early instars	
Day	Control	1 mg/l	5 mg/l	Control	1 mg/l	5 mg/l	Control	1 mg/l	5 mg/l	1 mg/l	5 mg/l	1 mg/l	5 mg/l	1 mg/l	5 mg/l
0*	6.0	3.9	4.1	3.2	2.2	2.5	2.8	1.7	1.6	–	–	–	–	–	–
1	3.2	0	0	1.9	0	0	1.4	0	0	100	100	100	100	100	100
2	2.2	0	0	0.8	0	0	1.5	0	0	100	100	100	100	100	100
3	2.4	0	0	1.3	0	0	1.1	0	0	100	100	100	100	100	100
4	2.3	0	0	1.1	0	0	1.2	0	0	100	100	100	100	100	100
5	2.3	0.1	0.0	1.6	0	0	0.8	0.1	0	91	100	100	100	70	100
6	0.9	0	0	0.7	0	0	0.3	0	0	100	100	100	100	100	100
7	0.6	0	0	0.4	0	0	1.7	0	0	100	100	100	100	100	100
8	0.4	0	0	0.1	0	0	0.3	0	0	100	100	100	100	100	100
11	0.2	0	0	0.1	0	0	0.2	0	0	100	100	100	100	100	100
15	0.4	0.5	0.2	0.0	0.1	0.0	0.3	0.4	0.2	0	7	0	100	0	0
19	0.1	0.3	0.2	0.0	0.1	0.1	0.0	0.3	0.1	0	0	0	0	0	0
23	0.2	0.5	0.3	0.1	0.2	0.1	0.1	0.3	0.3	0	0	0	0	0	0
26	0.4	0.7	0.8	0.1	0.2	0.3	0.3	0.5	0.5	0	0	0	0	0	0
28	0.6	0.7	0.7	0.2	0.3	0.3	0.4	0.4	0.4	0	0	0	0	0	0
33	1.4	1.6	1.6	0.4	0.6	0.7	1.1	1.1	0.9	0	0	0	0	0	0
35	0.4	1.1	1.7	0.2	0.6	0.9	0.1	0.6	0.7	0	0	0	0	0	0
37	0.6	1.1	0.6	0.3	0.6	0.2	0.3	0.5	0.4	0	0	0	0	0	0

* Day of application.

settings, are developed (Barbazan *et al.* 1997) and evaluated.

Our comparison of two different formulations, powder (PP/TP) and WDG, showed that the activity of the low potency WDG was slightly better than the activity of the high potency TP/PP. These results confirm the observation of Tianyun and Mulla (1999) that formulation technology can enhance the activity of products and that the WDG formulations are promising for control of disease vectors. We furthermore showed that under laboratory conditions the larvae of *A. gambiae* are more susceptible to the tested *Bs* formulations than to the *Bti* formulations, as has been reported from studies in natural breeding sites in the Democratic Republic of Congo (Karch *et al.* 1991), and Burkina Faso (Majori *et al.* 1987; Lacey *et al.* 1988).

Aedes and *Culex* larvae are supposed to be more susceptible to *Bti* and *Bs* products than *Anopheles* larvae (Porter *et al.* 1993; Charles & Nielsen-LeRoux 2000). But we found high sensitivity of *A. gambiae* to the formulations tested. In fact, when comparing our work with the data of Tianyun and Mulla (1999) who tested the same formulations (TP and WDG) on *C. quinquefasciatus* it is clear that *A. gambiae* is even more susceptible. The same holds for *Bti* WDG formulations against *Aedes aegypti* (LC_{50} 0.07 p.p.m.) when compared with *A. gambiae s.s.* (LC_{50} 0.02 p.p.m.). Our finding of the high sensitivity to *Bti*, on the other hand, corresponds well with those of Seyoum and Abate (1997) who reported similar sensitivities for *A. arabiensis*.

Results from the open field trials with *Bti* WDG showed that only a very low dosage of 200 g/ha (2700 ITU/mg) is required to effectively suppress late instars and the resulting pupae. This value corresponds well with the LC_{95} (0.2 p.p.m.) of the laboratory tests and represents the optimum effective dosage to control *A. gambiae* in western Kenya. Such low application dosages offer the possibility to keep operational costs low even if weekly treatments, caused by the absence of residual activity, have to be considered. This lack of residual effect of *Bti* has been reported previously (Das & Amalraj 1997). In the Democratic Republic of Congo, Karch *et al.* (1991) showed that larval populations began to recover 5–7 days after treatment at the latest, irrespective of the *Bti* concentration applied (2000–5000 ITU/l, in 0.1 m water depth). *Bs*, on the other hand, is expected to provide a greater residual larvicidal activity because of the longer persistence of the spores in the environment and their recycling potential in the gut of larvae after dying (Becker *et al.* 1995). This recycling can

lead to the control of several mosquito generations, although contradictory findings have been reported and seem to depend on formulation and application technology, species and densities thereof and environmental factors. Long residual activity of *Bs* formulations has mostly been shown in experiments where larvae were added at regular intervals and at comparatively high densities (Pantuwatana *et al.* 1989). Furthermore, investigations have mainly focused on *Culex* species in polluted water (Sutherland *et al.* 1989; Hougard 1990; Lago *et al.* 1991; Gunasekaran *et al.* 1996) where they occur in very high numbers (e.g. 80–160 larvae/dip). Nevertheless, even in *Culex* control, Karch *et al.* (1990) could not show a residual effect beyond 12–14 days. Repeated treatment over a short time interval may increase the duration of persistence (Karch *et al.* 1990, 1991) but complicates the logistics of control operations. Mulla *et al.* (1999), who tested the same *Bs* WDG formulation we did under field conditions in similar concentrations on *C. quinquefasciatus*, also confirmed quite variable results in longevity of the product between 1 and 4 weeks. In our study *Bs* WDG produced significant reduction of *Anopheles* and *Culex* larvae for up to 2 weeks, in spite of using concentrations of 25 and 50 times the LC_{95}. Much longer periods have been recorded for *Anopheles* control (S. Krause, personal communication) but low densities of larvae and solar inactivation of the product (Rojas *et al.* 2001) in our setting may have contributed to this reduced activity period.

Similar to work undertaken in the Democratic Republic of Congo (Karch *et al.* 1992), where retreatment took place at 15-day intervals, we propose a surface application regime of once every week for *Bti* and once every fortnight for *Bs* to achieve > 95% reduction on mosquito emergence from breeding sites.

Conclusions

The main malaria vectors in the *A. gambiae* complex are highly susceptible to the microbial control agents *B. sphaericus* and *B. thuringiensis* var. *israelensis*. The new WDG formulations are final formulations, ready for use in the field, and much easier to handle than traditional technical powder formulations. Compared with other ready-to-use formulations such as granules, WDG has the advantage of shipping higher potency material in lower volume and weight. Furthermore, the minimum effective dosages to kill 100% of the larval population in a habitat have shown to be extremely low and the products may therefore have great potential for inclusion in integrated vector management operations.

Acknowledgements

We thank Jackton Arija, Hassan Ali Akelo and their teams (ICIPE, Mbita insectary) for providing mosquitoes and George Sonye for field work assistance. The continued support from Prof. John Beier, Dr John Githure, Dr Steve Krause and Dr Robert Fusco towards this research programme is highly appreciated. We thank Valent Biosciences Corp. for funding support and provision of the formulations evaluated in this study, as well as GFS, Germany, for assistance in administration and research. BGJK is supported by NIH-ICIDR grant 5U19AI45511. The Kenya Pesticide Product Control Board approved this study through an experimental permit.

References

Barbazan P, Baldet T, Darriet F, Escaffre H, Djoda DH & Hougard JM (1997) Control of *Culex quinquefasciatus* (Diptera: Culicidae) with *Bacillus sphaericus* in Maroua, Cameroon. *Journal of the American Mosquito Control Association* 13, 263–269.

Barbazan P, Baldet T, Darriet F, Escaffre H, Djoda DH & Hougard JM (1998) Impact of treatments with *Bacillus sphaericus* on *Anopheles* populations and the transmission of malaria in Maroua, a large city in a savannah region of Cameroon. *Journal of the American Mosquito Control Association* 14, 33–39.

Becker N (1992) Community participation in the operational use of microbial control agents in mosquito control programmes. *Bulletin of the Society of Vector Ecology* 17, 114–118.

Becker N (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 & Rettich F (1994) Protocol for the introduction of new *Bacillus thuringiensis israelensis* products into the routine mosquito control program in Germany. *Journal of the American Mosquito Control Association* 10, 527–533.

Becker N, Zgomba M, Petric D, Beck M & Ludwig M (1995) Role of larval cadavers in recycling processes of *Bacillus sphaericus*. *Journal of the American Mosquito Control Association* 11, 329–334.

Blanco Castro SD, Martinez Arias A, Cano Velasquez OR, Tello Granados R & Mendoza I (2000) Introduction of *Bacillus sphaericus* strain-2362 (GRISELESF) for biological control of malaria vectors in Guatemala. *Revista Cubana de Medicina Tropical* 52, 37–43.

Breman JG (2001) The ears of the hippopotamus: manifestations, determinants and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene* 64, 1–11.

Chandre F, Darrier F, Manga L *et al.* (1999) Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bulletin of the World Health Organization* 77, 230–234.

Charles JF & Nielsen-LeRoux C (2000) Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. *Memorias Do Instituto Oswaldo Cruz* 95, 201–206.

Consoli RA, Santos BS, Lamounier MA *et al.* (1997) Efficacy of a new formulation of *Bacillus sphaericus* 2362 against *Culex quinquefasciatus* (Diptera: Culicidae) in Montes Claros, Minas Gerais, Brazil. *Memorias Do Instituto Oswaldo Cruz* 92, 571–573.

Curtis CF (1994) Should DDT continue to be recommended for malaria vector control? *Medical and Veterinary Entomology* 8, 107–112.

Das PK & Amalraj DD (1997) Biological control of malaria vectors. *Indian Journal of Medical Research* 106, 174–197.

Finney DJ (1971) *Probit Analysis*. Cambridge University Press, Cambridge.

Guillet P, Kurstak D, Philippon B & Meyer R (1990) Use of *Bacillus thuringiensis israelensis* for onchocerciasis control in West Africa. In: *Bacterial Control of Mosquitoes and Blackflies* (eds H de Barjac & DJ Sutherland) Rutgers University Press, New Jersey, pp. 187–199.

Gunasekaran K, Shriram AN, Elangovan A, Narayanan RJ & Balaraman K (1996) Efficacy of *Bacillus sphaericus* in different breeding habitats of *Culex quinquefasciatus*. *Southeast Asian Journal of Tropical Medicine and Public Health* 27, 622–627.

Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J & Coetzee M (2000) *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology* 14, 181–189.

Hays CW (2000) The United States Army and malaria control in World War II. *Parassitologia* 42, 47–52.

Hintze J (1997) *NCSS 6.0*. Microsoft Corporation, Seattle.

Hougard JM (1990) Formulation and persistence of *Bacillus sphaericus* in *Culex quinquefasciatus larval sites in tropical Africa*. In: *Bacteriological Control of Mosquitoes and Blackflies* (eds H de Barjac & DJ Sutherland). Rutgers University Press, New Brunswick, pp. 295–306.

Karch S, Monteny N, Jullien JL, Sinegre G & Coz J (1990) Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling. *Journal of the American Mosquito Control Association* 6, 47–54.

Karch S, Manzambi ZA & Salaun JJ (1991) Field trials with Vectolex (*Bacillus sphaericus*) and Vectobac (*Bacillus thuringiensis* (H-14) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. *Journal of the American Mosquito Control Association* 7, 176–179.

Karch S, Asidi N, Manzambi ZM & Salaun JJ (1992) Efficacy of *Bacillus sphaericus* against the malaria vector *Anopheles gambiae* and other mosquitoes in swamps and rice fields in Zaire. *Journal of the American Mosquito Control Association* 8, 376–380.

Killeen GF, Fillinger U & Knols BGJ (2002) Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malaria Journal* 1, 8.

Killeen GF, Fillinger U, Kiche I, Gouagna LC & Knols BGJ (2002) Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet Infectious Diseases* 2, 618–627.

Kitron U & Spielman A (1989) Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Reviews of Infectious Diseases* 11, 391–406.

Kroeger A, Dehlinger U, Burkhardt G, Atehortua W, Anaya H & Becker N (1995a) Community based dengue control in Columbia: people's knowledge and practice and the potential contribution of the biological larvicide *Bti* (*Bacillus thuringiensis israelensis*). *Tropical Medicine and Parasitology* 46, 241–246.

Kroeger A, Horstick O, Riedl C, Kaiser A & Becker N (1995b) The potential for malaria control with the biological larvicide *Bacillus thuringiensis israelensis* (*Bti*) in Peru and Ecuador. *Acta Tropica* 60, 47–57.

Lacey LA, Lacey CM, Peacock B & Thiery I (1988) Mosquito host range and field activity of *Bacillus sphaericus* isolate 2297 (serotype 25). *Journal of the American Mosquito Control Association* 4, 51–56.

Lago GM, Perez MC, Figueroa AM & Sonzalez FAC (1991) Results of a pilot application of the biolarvicide *Bacillus sphaericus* to larval habitats of mosquitoes in Santa Cruz del Norte municipality (La Habana Province). *Revista Cubana de Medicina Tropical* 43, 39–44.

Lengeler C (2001) Insecticide-treated bednets and curtains for preventing malaria (Cochrane Review). *The Cochrane Library* 4, Oxford (Update Software), pp. 1–70.

Lyimo EO & Takken W (1993) Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae* females in Tanzania. *Medical and Veterinary Entomology* 7, 328–332.

Majori G, Ali A & Sabatinelli G (1987) Laboratory and field efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* against *Anopheles gambiae s.l. and Culex quinquefasciatus* in Ouagadougou, Burkina Faso. *Journal of the American Mosquito Control Association* 3, 20–25.

Minakawa N, Mutero CM, Githure JI, Beier JC & Yan G (1999) Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *American Journal of Tropical Medicine and Hygiene* 61, 1010–1016.

Mnzava AE, Rwegoshora RT, Tanner M, Msuya FH, Curtis CF & Irare SG (1993) The effects of house spraying with DDT or lambda-cyhalothrin against *Anopheles arabiensis* on measures of malarial morbidity in children in Tanzania. *Acta Tropica* 54, 141–151.

Mulla MS, Norland RL, Fanara DM, Darwazeh A & McKean D (1971) Control of chironomid midges in recreational lakes. *Journal of Economic Entomology* 64, 300–307.

Mulla MS, Su T, Thavara U, Tawatsin A, Ngamsuk W & Pan-Urai P (1999) Efficacy of new formulations of the microbial larvicide *Bacillus sphaericus* against polluted water mosquitoes in Thailand. *Journal of Vector Ecology* 24, 99–110.

Mutero CM, Ouma JH, Agak BK, Wanderi JA & Copeland RS (1998) Malaria prevalence and use of self-protection measures against mosquitoes in Suba district. *East African Medical Journal* 75, 11–16.

Nabarro DN & Tayler EM (1998) The 'Roll Back Malaria' campaign. *Science* 280, 2067–2068.

Pantuwatana S, Maneeroj R & Upatham ES (1989) Long residual activity of *Bacillus sphaericus* 1593 against *Culex quinquefasciatus* larvae in artificial pools. *Southeast Asian Journal of Tropical Medicine and Public Health* 20, 421–427.

Porter AG, Davidson EW & Liu JW (1993) Mosquitocidal toxins of bacilli and their genetic manipulation for effective biological control of mosquitoes. *Microbiological Reviews* 57, 838–861.

Ragoonanansingh RN, Njunwa KJ, Curtis CF & Becker N (1992) A field study of *Bacillus sphaericus* for the control of culicine and anopheline mosquito larvae in Tanzania. *Bulletin of the Society of Vector Ecology* 17, 45–50.

Ravoahangimalala O, Thiery I & Sinegre G (1994) Rice field efficacy of Deltamethrin and *Bacillus thuringiensis israelensis* formulations on *Anopheles gambiae s.s.* the Anjiro region of Madagascar. *Bulletin of the Society of Vector Ecology* 19, 169–174.

Regis L, Oliveira CM, Silva-Filha MH, Silva SB, Maciel A & Furtado AF (2000a) Efficacy of *Bacillus sphaericus* in *control of the filariasis vector Culex quinquefasciatus* in an urban area of Olinda, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 94, 488–492.

Regis L, Silva SIB & Melo-Santos MAV (2000b) The use of bacteria larvicides in mosquito and black fly control programmes in Brazil. *Memorias Do Instituto Oswaldo Cruz* 95, 207–210.

Reymond M (1985) Presentation d'un programme d'analyse log-probit pour microordinateur. *Cahiers ORSTOM, Série Entomologie Médiciale et Parasitologie* 22, 117–121.

Roberts DR, Manguin S & Mouchet J (2000) DDT house spraying and re-emerging malaria. *Lancet* 356, 330–332.

Rodrigues IB, Tadei WP & Dias JM (1998) Studies on the *Bacillus sphaericus* larvicidal activity against malarial vector species in Amazonia. *Memorias Do Instituto Oswaldo Cruz* 93, 441–444.

Rodrigues IB, Tadei WP & Dias JM (1999) Larvicidal activity of *Bacillus sphaericus* 2362 against *Anopheles nuneztovari, Anopheles darlingi* and *Anopheles braziliensis* (Diptera, Culicidae). *Revista Do Instituto Medicina Tropical de Sao Paulo* 41, 101–105.

Rojas JE, Mazzarri M, Sojo M & Garcia AG (2001) Effectiveness of *Bacillus sphaericus* strain 2362 on larvae of *Anopheles nuneztovari*. *Investigacion Clinica* 42, 131–146.

Romi R, Ravoniharimelina B, Ramiakajaro M & Majori G (1993) Field trials of *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* (strain 2362) formulations against *Anopheles arabiensis* in the central highlands of Madagascar. *Journal of the American Mosquito Control Association* 9, 325–329.

Rozendaal JA (1997) *Vector Control. Methods for Use by Individuals and Communities*. WHO, Geneva, pp. 1–398.

Schnetter W, Engler S, Morawcik J & Becker N (1981) Wirksamkeit von *Bacillus thuringiensis* var. *israelensis* gegen Stechmuecken und Non-Target Organismen. *Mitteilungen der Deutschen Gesellschaft fuer Allgemeine und Angewandte Entomologie* 2, 195–202.

Seyoum A & Abate D (1997) Larvicidal efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* on *Anopheles arabiensis* in Ethiopia. *World Journal of Microbiology and Biotechnology* 13, 21–24.

Shousha AT (1948) Species-eradication. The eradication of *Anopheles gambiae* from Upper Egypt, 1942–1945. *Bulletin of the World Health Organisation* 1, 309–353.

Skovmand O & Sanogo E (1999) Experimental formulations of *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* against *Culex quinquefasciatus* and *Anopheles gambiae* (Diptera: Culicidae) in Burkina Faso. *Journal of Medical Entomology* 36, 62–67.

Soper FL & Wilson DB (1943) *Anopheles gambiae in Brazil*. The Rockefeller Foundation, New York.

Sutherland DD, McNelly JJ & Hansen JA (1989) Evaluation of granular *Bacillus sphaericus* to control *Culex* in sewage treatments ponds in Cape May. *New Jersey Mosquito Control Association* 76, 84–90.

Tianyun Su & Mulla MS (1999) Field evaluation of new water-dispersible granular formulations of *Bacillus thuringiensis* ssp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquitoes in microcosms. *Journal of the American Mosquito Control Association* 15, 356–365.

Trape JF (2001) The public health impact of chloroquine resistance in Africa. *American Journal of Tropical Medicine and Hygiene* 64, 12–17.

Utzinger J, Tozan Y & Singer BH (2001) Efficacy and cost-effectiveness of environmental management for malaria control. *Tropical Medicine and International Health* 6, 677–687.

WHO (1996) *Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides*. CTD/WHOPES/IC/96.1, WHO, Geneva, pp. 1–69.

WHO (1999) International programme on chemical safety (IPCS): microbial pest control agent *Bacillus thuringiensis*. *Environmental Health Criteria* 217, 1–105.