Journal of Vector Ecology 22(1): 43-51

Efficacy of a Granular Formulation of *Bacillus sphaericus* against *Culex quinquefasciatus* and

O. Skovmand[1] and S. Bauduin[2]

Danish Bilharzia Laboratory, Jaegersborg Alle 1D, DK-2920 Charlottenlund, DENMARK
[1]Current Address and [2]Orstom-LIN, 911 Ave. Agropolis, B.P. 5045,
F-34032 Montpellier Cedex 1, FRANCE

Received 1 August 1996; Accepted 26 November 1996

ABSTRACT: The efficacy of a sustained released granular formulation of *Bacillus sphaericus* strain 2362 was compared to a flowable concentrate in containers, cesspools, and water ponds. Duration of control was dependent on formulation, dosage, exposure to sun, site, recycling, and target mosquito larvae. In a series of container tests with repeated additions of fourth-instar *Culex quinquefasciatus* larvae exposed to 0.3 or 3.0 g/m² urban cesspools were not removed, more than 95 percent control was obtained for two and four days

to the surface. Settling of spores and toxins are among the factors that limited the effectiveness of the application (Davidson et al. 1984). Nicolas et al. (1987b) and Pailey et al. (1987) demonstrated that the number of spores and vegetative cells declined in the upper water layer in cesspools and increased at the bottom. The relatively slow decrease at the upper water layer combined with a high application rate (10 g/m^2) would then explain the residual effect.

However, in many cases, and especially in areas exposed to the sun, the residual effect is very short (Lacey et al. 1988b, Mulla 1986) and the product has to be reapplied at least weekly to control larvae (Aranda and Eritja 1992, Sinegre et al. 1993). Sustained release formulations may prolong the residual effect, but are

min. in water with a droplet of Tween 80 after shaking. During this period, granule samples were homogenized several times with a Kinematic homogenizer with a Polytron PTA 20S rod for 30 sec. to break up the granules. All samples were homogenized with a Waring blender after dilution to 400 ml. No detergent was added to the fluid samples. After further dilution with tap water, 10 ml was transferred to three beakers with 90 ml dechlorinated tap water (in two tests: sewage water) with 20 L_4 larvae. The stock suspension was diluted to 50 percent, 10 ml was transferred to the next three beakers, etc. to make six to seven dilutions. An LC_{50} was calculated using a log-probit program with working probits as described by Finney (1971). Potencies of

[redacted]

was cautiously added to compensate for evaporation. The tests were carried out in the French Organisation for Cooperation in Development and Research (ORSTOM) facilities in Montpellier, southern France. It did not rain during the period of the outdoor experiments. In the container studies, 50 *Cx. quinquefasciatus* L_4 were added with fixed daily intervals and live larvae were removed after two days exposure. Larvae were removed from the surface when they appeared to breath with sterile pipettes not to resuspend sediment product. Cadavers were not removed. Larvae not found were counted as dead. In some tests, surviving larvae were brought to the laboratory and their mortality was followed

per site. This method was preferred to one comparing the numbers of larvae in the sites before and after treatment, because of some cases of migrating larvae from untreated neighboring chambers. First instars were not included since their life span is shorter than the killing rate of the products. Coexisting *Culex decens* (Theobald) larvae were not included.

In the Senegal study, the test sites consisted of 18 unconnected holes (5-25 m^2) dug into a dry river basin, thus excluding immigration of larvae between basins. Effect was measured as mean number of *Anopheles gambiae* larvae caught with a 200 ml tray sampling along the edge of the ponds (3 samples in small ponds,

SPH88 is reported to contain $1.3x10^{11}$ spores/g. The granule and the flowable concentrate were applied at 0.3 and 3.0 g/m^2 in the field test and in the container test.

Bioassays of flowable concentrate of the granule yielded LC_{50} values depending on water type: 1.2 µg Spherimos/g sewage water and 1.3 µg granulate/g sewage

($1x10^6$ spores/g along the edge where most cadavers were found) and to $1.8x10^3$ spores/g where larvae were not added weekly.

The mean number of larvae in pretreatment counts in the cesspool in Cameroon was 85 larvae/3 dips (range 9-900). Analysis of variance of the control sites showed

TABLE 2. Mean number of dead L_4 with a flowable concentrate and a granular formulation in 75 l indoor containers with sewage water and lid on. Mean number of dead larvae are given for three containers per group (two in control). Fifty L_2 added to all containers weekly except for one treatment group, where larvae were added day 34.

Days After Application	Control	Fluid Conc. 0.3 g/m² Larvae Applied Weekly	Fluid Conc. 0.3 g/m² Larvae Applied Day 34
0	5[1]	44.3 ± 7.4	No larvae added
5	5[1]	47.7 ± 3.2	No larvae added
11	1.5	47.3 ± 3.8	No larvae added
16	0	50	No larvae added
26	0.5	50	No larvae added
34	1	50	6.3 ± 2.5

[1]*Notonecta* sp was found in one control container with initial high control mortality and was removed on day 7.

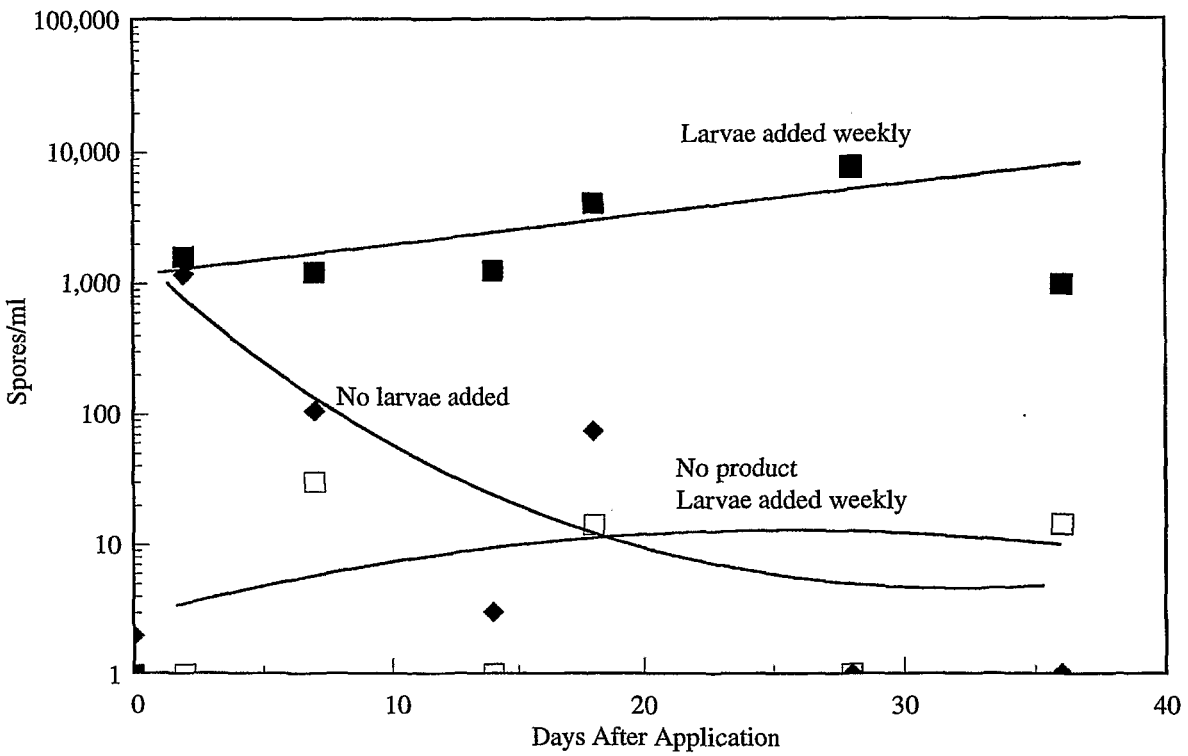


Figure 1. Mean number of *B. sphaericus* spores per ml at the surface of containers treated with the flowable concentrate and with or without weekly addition of larvae.

TABLE 3. Mean number of larvae per treatment in cesspools treated at two dosages. Numbers with the same indexes are not significanty different (P<0.05; a,b: analysis of variance per day across products, x,y,z: variance analysis per treatment group across days).

Treatment	Number of sites	Pre-treatment	Day 2	Day 5	Day 8	Day 16
Fluid Conc. 0.3 g/m^2	5	110^{ax}	0.8^{ay}	4.8^{az}	21.2^{ax}	67.6^{ax}
Fluid conc 3.0 g/m^2	10	127^{ax}	1.0^{ay}	1.3^{aby}	4.3^{by}	14.4^{bz}
Gran, Big 0.3 g/m^2	10	34^{ax}	1.9^{ay}	5.4^{az}	9.8^{by}	6.9^{byz}
Gran, Big 3.0 g/m^2	10	108^{ax}	0.1^{ay}	0.4^{by}	0.3^{bx}	5.1^{by}
Gran, Small 3.0 g/m^2	5	52^{ax}	0.2^{ay}	0.2^{ay}	3.4^{bz}	5^{by}
Control	5	65^{ax}	37^{x}	21^{x}	22^{x}	45^{x}

with Spherimos at the high dosage. In the third, the number of larvae increased above pretreatment levels; 3.0 g/m^2 of the flowable concentrate gave control above 90 percent for six days only and no control at all after 15 days (Fig. 2). At this time, there was still 50 percent control by the granule at the low dosage and 100 percent control at the high dosage.

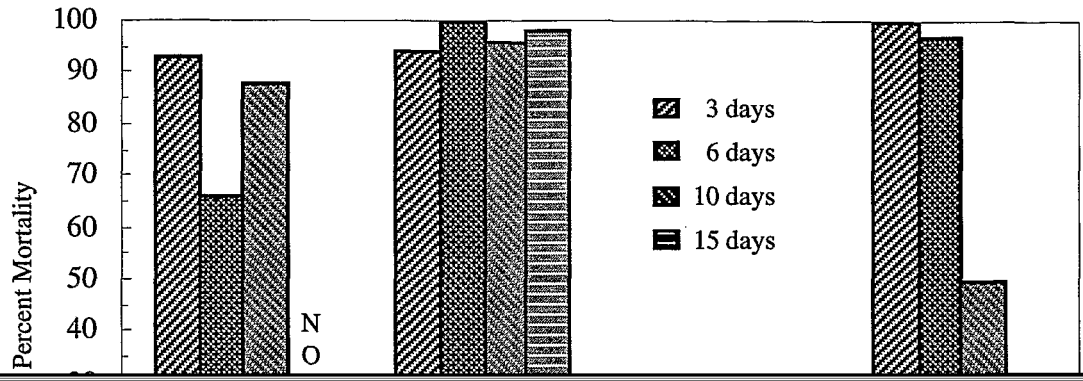
DISCUSSION

A granular formulation of *B. sphaericus* was compared to a flowable concentrate in trials in southern France and in West Africa. The container trials revealed a major influence of sun exposure on the control effect on both formulations (TABLE 1), at least in southern France in spring and summer where the UV-irradiation is high and much higher than in Africa south of Sahara (Monteny, 1990). Without sun exposure, a single application of 0.3 g/m^2 Spherimos initially resulted in 90 percent control increasing to 100 percent for five weeks when larvae were added weekly (TABLE 2). Without the weekly addition of larvae, control declined to 15 percent after the five weeks. Spore counts at the bottom and surface of containers confirmed the amplification of *B. sphaericus* at the water surface where larvae were added compared to the decline where they were not (Fig. 1). This demonstrates the recycling of *B. sphaericus* through host larvae in polluted water as demonstrated previously for mosquito larvae in clean or distilled water (Kramer 1990, Becker et al. 1995, Correa and Yousten 1995). Contrary to that found by Nicolas et al. (1987b), we found black mosquito cadavers floating at the surface of the sewage water for several days, which may explain why we obtained effect of recycling. Bacterial counts at the bottom confirmed sedimentation as also reported in other studies (Davidson et al. 1984, Nicolas et al. 1987b).

Fourth-instar *Cx. quinquefasciatus* larvae given a lethal dosage of *B. sphaericus* produced very variable number of cells (10^3-10^5) per cadaver. Davidson et al (1984) reported 10^5 to 10^6 spores/cadaver for *Cx. tarsalis* and Paily et al (1987) found 2.5 x 10^3 spores/cadaver for *Cx. tritaeniorhynchus*. Provided all larvae died and the bacterium proliferated in the cadavers, 50 larvae per week for five weeks would add 2.5 x 10^7 to 10^8 spores compared to the 2.5 x 10^7 spores applied with the product. In comparison, 1.3 x 10^9 of spores/g was estimated in one mm mud at the bottom or more than 10 times the bacteria added or reproduced in mosquito larvae. That may indicate that *B. sphaericus* not only recycled in the cadavers, but was multiplying in the sewage water or in the mud also.

Sustained effect due to recycling in cadavers is counteracted by settling and other inactivations of spores and crystals and requires a continuous presence of larvae as provided in this study. On account of this, Arrendondo-Jimenez et al. (1990) found longer residual activity in field tests where the control was below 100 percent.

When sun exposed containers were treated, the


100
90
80
70
60
50
40
Percent Mortality
N
O
3 days
6 days
10 days
15 days

following. In our study on *An. gambiae*, the high dosage of the granule and of the fluid concentrate was 500,000 ITU/m^2 and 750,000 ITU/m^2, respectively, and the low dosage 10 times lower. Testing on *An. stephensi* Liston, Kumar et al. (1994) reported continuous control by weekly application of powder of 450.000 ITU/m^2 in covered water tanks, while tests with *An. gambiae* at dosages from 2,500 to 2,500,000 ITU/m^2 in sun exposed areas gave at the most two days control (Darriet et al. 1985, Nicholas 1987a, Karch et al. 1991, Karch et al. 1992). Very short effects or practically no reduction was observed for other anopheline species (Balaraman et al. 1987, Lacey et al. 1988b), which might be due to lower sensitivity. *Anopheles quadrimaculus* Say is about 20 times less susceptible than *An. albimanus* Wiedemann (Mulla 1986), and *An. gambiae* is three times more susceptible than *An. albimanus*. The long control obtained in this study is not due to a higher dosage than in other studies.

Acknowledgments

The authors wish to thank Philip Barbazan and Vincent Roger for identifying field sites in Cameron and Senegal, respectively, and providing laboratory facilities to support the field test; Patchoke Salomon for helping with the field tests in Cameron; and Lawrence Lacey for valuable comments on the manuscript. The study was financed by a Danida Research Grant 104.Dan8/659 and carried out in cooperation between the Danish Bilharzia Laboratory and ORSTOM, Montpellier, Laboratoire de Lutte contre les Insectes Nuisible.

REFERENCES CITED

Aranda, C. and R. Eritja. 1992. Laboratory and field efficacy of *Bacillus sphaericus* 2362 against *Culex pipiens* in the river Llobregat delta (Barcelona, Spain). Bull. Soc. Vector Ecol. 17: 41-44.

de Barjac, H. 1985. Standardised test for the determination of the susceptibility of mosquito larvae to bacterial toxins. WHO Report VBC/ECV/EC/85.25, 5 pp.

de Barjac, H. and I. Larget-Thiery. 1984. Characteristics of IPS 82 as standard for biological assay of *Bacillus thuringiensis* H-14 preparations. WHO Report VBC/84.892, 10 pp.

Becker, N., M. Zgomba, D. Petric, M. Beck, and M. Ludwig. 1995. Role of larval cadavers in recycling processes of *Bacillus sphaericus*. J. Am. Mosq. Contr. Assoc. 11: 329-334.

Correa, M. and A. A. Yousten. 1995. *Bacillus sphaericus* spore germination and recycling in mosquito larval cadavers. J. Invertebr. Pathol. 66: 76-81.

Dahl, C. 1988. Control potentials in feeding mechanisms of mosquito larvae. Bull. Soc. Vector Ecol. 13: 295-303.

Darriet, F., L. Nicolas, and J. M. Hougard. 1985. Evaluation de l'ativite au laboratoire et sur le terrain de *Bacillus sphaericus* 2362 sur *Anopheles gambiae* s.l. Giles, 1902 en Afrique de l'Ouest (Burkina-Faso). ORSTOM Report No 27/ipr/rap/85, pp 1-10.

Davidson, E. W., M. Urbina, J. Payne, M. S. Mulla, H. Darwazeh, H. T. Dulmage, and J. A. Correa. 1984. Fate of spores used as a larvicide in the aquatic environment. Appl. Environ. Microbiol. 47: 125-129.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, pp. 100-121.

Hougard, J.-M., R Mbentengam, L. Lochouarn, H. Escaffre, F. Darriet, P. Barbazan, and D Quillévéré. 1993. Lutte contre *Culex quinquefasciatus* par *Bacillus sphaericus*: résultats d'une campagne d'Afrique équatoriale. WHO Bull. 71: 367-375.

Karch, S., Z. A. Manzambi, and J. J. Salaun. 1991. Field trials with Vectolex (*Bacillus sphaericus*) and

Kumar, A., V. P. Sharma, P. K. Sumodan, D. Thavaselvam, and R. H. Kamat. 1994. Malaria control utilising *Bacillus sphaericus* against *Anopheles stephensi* in Panaji, Goa. J. Am. Mosq. Contr. Assoc. 10: 534-539.

Lacey, A.L. 1990. Persistence and formulation of *Bacillus sphaericus*, Pp. 284-294 *in* Bacterial Control of Mosquitoes and Blackflies. (H. de Barjac and D. J. Sutherland, eds.). Rutgers University Press, New Brunswick, USA.

Lacey, L. A., M. J. Urbina, and C. M. Heitzman. 1984. Sustained release formulation of *Bacillus sphaericus* and *Bacillus thuringiensis (H-14)* for control of container breeding *Culex quinquefasciatus*. Mosq. News 44: 26-32.

Lacey, L. A., C. M. Lacey, B. Peacock, and E. Thierry. 1988a. Mosquito host range and field activity of *Bacillus sphaericus* isolate 2297 (serotype 25). J Am. Mosq. Contr. Assoc. 4: 51-56.

Efficacy of *Bacillus sphaericus* 2362 against larvae of *Anopheles gambiae* under laboratory and field conditions in West Africa. Med. Vet. Entomol. 1: 157-162.

Nicolas, L., J. Dossou-Yovo, and J. M. Hougard. 1987b. Persistence and recycling of *Bacillus sphaericus* 2362 spores in *Culex quinquefasciatus* breeding sites in West Africa. Appl. Microbiol. Biotechnol. 25: 341-345.

Pailey, K. P., A. K. Virmani, and S. Jayachandran. 1987. Utility of *Bacillus sphaericus* in controlling *Culex tritaeniorhynchus* breeding. FEMS Microbiol. Ecol. 45: 313-318.

Sinegre, G., M. Babinot, G. Vigo, G. Gaven, and J. L. Julien. 1993. *Bacillus sphaericus* et demoustication urbaine. Entente Interdepartementale pour la demoustication du Littoral Mediterraneen, Document E.I.D.L.M. no 62.

Singer, S. 1973. Insecticidal activity of recent bacterial

Pole 2
D 10

Efficacy of a Granular Formulation of *Bacillus sphaericus* against *Culex quinquefasciatus* and *Anopheles gambiae* in West African Countries

O. Skovmand[1] and S. Bauduin[2]

Danish Bilharzia Laboratory, Jaegersborg Alle 1D, DK-2920 Charlottenlund, DENMARK

[1]Current Address and [2]Orstom-LIN, 911 Ave. Agropolis, B.P. 5045, F-34032 Montpellier Cedex 1, FRANCE

Journal of Vector Ecology
Society for Vector Ecology
Vol. 22, No. 1: 43-51, June, 1997

Fonds Documentaire ORSTOM
010011203

Fonds Documentaire ORSTOM
Cote : B*11203 Ex:1