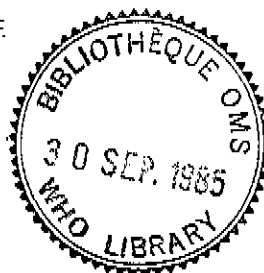




CONSULTATION ON THE DEVELOPMENT
 OF BACILLUS SPHAERICUS AS A
 MICROBIAL LARVICIDE

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Geneva, 7-11 October 1985

FIELD EVALUATION OF VARIOUS B. SPHAERICUS
 STRAINS ON CULEX QUINQUEFASCIATUS LARVAE IN TROPICAL AFRICA

by

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1. INTRODUCTION

In Africa the urban breeding places of Culex quinquefasciatus essentially consist of places where polluted water collects such as cesspools, septic tanks, latrines and gutters. These sites are well defined and easy to catalogue, so that the control of this mosquito is primarily based on the use of larvicides.

Resistance of C. quinquefasciatus to many chemical insecticides makes it necessary to evaluate new control methods such as insect growth regulators or biological control agents. Of the latter, Bacillus thuringiensis H-14 and various strains of Bacillus sphaericus have undergone field trials, particularly in Ivory Coast, with financial support from the Special Programme for Research and Training in Tropical Diseases (TDR).

While the residual effect of B. thuringiensis H-14 has proved to be less than 10 days whatever concentration is used (Hougard et al., 1983; Sudomo et al., 1981), this limitation does not apply to B. sphaericus which has accordingly been subjected to more detailed studies: entomological evaluation, bacteriological studies and biological titration of different strains.

2. ENTOMOLOGICAL EVALUATION

Trials conducted in Ivory Coast with strain 1593-4 showed that the residual effect of this bacterium increases with concentration and exceeds 3 weeks at a concentration of 10 g/m² (Hougard et al., 1985). These results confirm those obtained by Mulligan et al. (1980) in California with the same strain.

A formulation (concentrated suspension) of strain 2362 (BSP T), tested in cesspools in Ivory Coast at a concentration of 10 g/m², suppressed the preimaginal population for 5 weeks; pupae made their reappearance after 6 weeks. In some of the cesspools treated with strain 1593 it was noted that a few days or a few weeks after the breeding sites became recolonized dead larvae reappeared on the surface of the water and live larvae were present in greater numbers. Analysis of the dead larvae showed they were infected by spores of B. sphaericus belonging to the same serotype as those used in the treatment (flagellar agglutination technique of Barjac et al., 1980).

Larger-scale trials have been carried out in Dar-es-Salaam (Tanzania) with the concentrated suspension BSP 1.

If the results confirm those obtained in Ivory Coast it is planned to treat over 80 latrines and septic tanks with a concentration of 10 g/m² for one year, at the rate of one

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treatment cycle every 6 weeks.

This experiment is interesting inasmuch as a formulation of B. sphaericus can be produced industrially, and could be considered for operational use in a focus of bancroftian filariasis.

3. BACTERIOLOGICAL STUDIES

Parallel with the entomological studies, the water of cesspools treated with strain 2362 (BSP I) was subjected to bacteriological tests at Bouaké (Ivory Coast). The spore counts were performed by spreading samples, taken from the breeding sites at different depths, on MBS agar medium with 0.01% streptomycin sulfate. These tests brought the following facts to light (Nicolas & Hougard, 1985):

- rapid and homogeneous diffusion of the spores throughout the entire cesspool less than 6 hours after treatment;
- very slow sedimentation of the spores: at a concentration of 10 g/m² the number of spores per ml at the surface drops from about 10⁴ at the time of treatment to just over 10² after 5 weeks (when the larvae start to reappear). 10² spores/ml seems to be the minimum number that permits complete control of the C. quinquefasciatus population, and this confirms the results of Davidson et al. (1984) and of Mulla et al. (1984);
- recycling of the bacterium in the dead mosquito larvae, also demonstrated by Davidson et al. (loc. cit.), Des Rochers & Garcia (1984) and Silapanuntakul et al. (1983). At the Bouaké laboratory C. quinquefasciatus larvae were placed in contact with a suspension of BSP I at a concentration of 2 x 10⁵ spores/ml for quarter of an hour, then kept under observation in untreated water: 72 hours after ingestion the number of spores in the dead larvae increased by a factor of 1000, rising from 3 x 10² spores/larva after quarter of an hour of contact time to 3 x 10⁵ spores/larva. This result is obtained both in distilled water and in polluted water.

Work is in progress to see whether this recycling also occurs in situ and to study what influence pollution factors may have on this phenomenon.

4. BIOLOGICAL TITRATION

Under the WHO Programme for evaluating different samples of B. sphaericus produced by industry and other research centres, we have so far received 14 such samples belonging to strains 2362, 2297 and 1593. Biological titration of the samples was performed on young stage III larvae from a local strain of C. quinquefasciatus. The results of these tests show that, as for B. thuringiensis H-14, the culture conditions and culture medium seem to play a predominant role in the efficacy of the products. With strain 2362, for example, the LC₅₀ after 48 hours varies from 0.0013 mg/litre to 0.5 mg/litre depending on the origin of the sample.

In view of these results and the inadequate quantities of product available in some of the batches received, only three of the samples, with efficacy comparable to the reference product SPH 84, will undergo small-scale evaluation in the field: 2362 (Dulmage), 2297 (Dulmage) and ABC 6184, an unknown strain (Abbott).

5. CONCLUSION

Whether or not recycling takes place in polluted water, the use of a formulation of B. sphaericus against C. quinquefasciatus in urban areas already seems operationally feasible. For example, at Dar-es-Salaam (Tanzania), with a 6-week treatment cycle at 10g/m², the concentrated suspension BSP I (strain 2362) could beneficially replace chlorpyrifos (Dursban) which is currently used by the city's sanitation service: in view of the resistance of C. quinquefasciatus to this compound the interval between treatments has been reduced over the last 10

years from 12 weeks (Bang et al., 1975) to 15 days (Curtis & Pasteur, 1981; Curtis et al., 1984).

As with B. thuringiensis H-14 used against blackfly larvae by the Onchocerciasis Control Programme (OCP) in West Africa, it is to be hoped that industry will be able to maintain the quality of preparations such as BSP I so that their use can be extended to other foci of bancroftian filariasis and to other African cities where C. quinquefasciatus represents a major hazard.

Moreover, new growth regulator compounds currently being tested in Ivory Coast are giving very encouraging results, with a residual effect of over 3 months in polluted waters (Doannio et al., 1985). A new treatment strategy could thus be considered, whereby these compounds are rotated with B. sphaericus.

Apart from the improvement in the performance of the various B. sphaericus preparations used as conventional larvicides (increase in residual effect, search for more toxic strains), studies should also be continued in order to find the optimum conditions for the recycling of this bacterium which will ensure full and lasting suppression of the preimaginal mosquito population.

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