BACILLUS THURINGIENSIS H14, A BIOCONTROL AGENT FOR ONCHOCERCIASIS CONTROL IN WEST AFRICA

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INTRODUCTION

Human onchocerciasis or river blindness control in Africa is actually based on the use of insecticides which kill the larval stage of the vector of the *Simulium damnosum* complex. The World Health Organization in 1974 started a vast control programme (O.C.P.) in seven West African countries. The programme covers an area of 764.000 km2 and 18.000 km of river system have to be treated each week for a period of twenty years. These treatments consist of aerial spraying of insecticides in the breeding sites. During dry season (low water) each breeding site must be treated individually whereas at high water (rainy season) treatment points are much more spaced. From 1974 to 1980, the use of an organophosphate compound, temephos, gave very good results in the control of *S. damnosum* complex populations with a tolerable effect on the equilibrium of non-target organisms in treated rivers. In 1980 resistance to temephos occurred in the *Simulium soubrense Simulium sanctipauli* pair, vector of onchocerciais in forest area (Guillet *et al.*, 1980). Another organophosphate compound, chlorphoxim, was used as an alternative but resistance developped to this compound also and spread out rapidly (Kurtak *et al.*, 1982). Recently cross resistance to several organophosphate compounds suitable in the context of this programme has been demonstrated (Guillet, Kurtak, unpublished data). The prospects of using other chemical insecticides are actually very limited because of their relative toxicity to riverine populations, non-target organisms or their low level of efficacy for the larvae of *S. damnosum* complex. In this context, *Bacillus thuringiensis* H14 appears to be not as an elegant solution for the future but as the only alternative available at present for the continuation of larviciding in the zones where resistance has occurred.

I. THE OPERATIONNAL USE OF *B. THURINGIENSIS* H14 IN WEST AFRICA

The formulation presently used is Teknar^{R} SAN 402 I (Sandoz). Among the different *B.t.* H14 formulations tested in rivers, Teknar represents the best compromise between the level of efficacy for *S. damnosum* complex larvae and the physical characteristics which enable its use in aerial operationnal treatments. The modalities of its use have already been described (dosage : 1.6 mg/l/10 mn, prior dilution of the formulation with 20 % water, application by helicopter equiped with nozzles or rapid release system, Guillet *et al.*, 1982 A, Cheke 1981 unpublished data). The stability of the pure or diluted formulation is remarkable since no loss of activity is noticeable after 16 months storage of the drums in the open sun in West Africa (Guillet *et al.*, 1982 B). The use of Teknar has however a serious logistic drawback because the quantities to be applied are 4 to 8 times more important than that of temephos and its effective "carry" is much less inferior. In a preliminary trial by O.C.P. in the Ivory Coast on a river with a very large discharge (457 m3/s) a carry of about 20 km was recorded (Lacey *et al.*, 1981) as compared to 50 to 60 km with temephos. However trials carried out subsequently in the O.C.P. have shown that this "carry" of 20 km is the maximum that can be expected with this formulation. Teknar is thus suitable for treat-

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ments during dry season when the discharges are low and the carry is consequently very limited whatever the formulation of insecticide used. On the contrary, its use in the rainy season because of the larger quantities which have to be applied and the number of treatment points is not realistic at present from practical and economical point of view. New batches of Teknar having an increased delta endotoxin content have been recently tested and found to be 2 to 4 times more effective (Guillet *et al.*, 1982 C, Ocran and Agoua, pers. comm.). When these formulations become commercially available, it should be possible to use *B.t.* H14 in the rainy season and eventually to extend its use in the savannah zones as an alternative to temephos to limit the possible spread of resistance to organophosphates into savannah populations of *S. damnosum* complex.

II. PROBLEMS ASSOCIATED WITH IMPROVEMENT OF *B. THURINGIENSIS* H14 BLACKFLY LARVICIDES

Generally the efficacy of blackfly larvicides is closely related to the formulation (Jamnback and Means, 1968 ; Guillet and Escaffre, 1979). It is the same for *B.t.* H14 formulations of which the efficacy vis-à-vis *S. damnosum* complex larvae does not necessarily depend on the delta endotoxin content expressed in terms of I.T.U. *A. aegypti*/mg. For a given formulation efficacy varies proportionnally with the delta endotoxin content (Table I). On the other hand from one formulation to another, the ratio *A. aegypti* biological titre/LC 50 *S. damnosum* can vary considerably (up to 200 times) indicating that for certain formulations there is no relation at all between *A. aegypti* titre and toxicity to blackfly larvae (table II). This disparity can be explained generally by three main factors : the active ingredient itself (parasporal crystals), blackfly larvae and their feeding behaviour and lastly the formulations.

II. 1. Toxicity of *B.t.* H14 crystals to blackfly larvae :

When *B.t.* H14 is tested on mosquito and blackfly larvae under comparable conditions (contact 24 h in the lab with distilled water) one can note a very satisfactory concordance between the *A. aegypti* and *S. damnosum* titres (table III). However when the same products are tested in troughs under more natural conditions this concordance no longer exists (table III). In these conditions, with an exposure period of 10 mn and an intestinal transit of 2 to 15 mn the larval mortality of blackfly larvae no longer varies proportionnally with the endotoxin content. It is probable that a proportion of toxin is not digested. If the retention time of the *B.t.* H 14 in the gut is artificially increased by inhibiting feeding immediately after treatment (Gaugler and Molloy, 1980) or by paralyzing the larvae with sublethal doses of pyrethroids its toxicity is considerably increased. The retention time of *B.t.* H14 crystals in the gut of *A. aegypti* larvae during bioassays where no food is provided is considerably longer than in blackfly larvae under natural conditions. It is not excluded that at different stages of the growth of crystals or the preparation of the products the toxin can be more or less digestible (membrane formation, presence

of proteases ...). This could be manifested by a variation in toxicity to blackfly larvae which may not be detected in the bioassay with *A. aegypti*. The growth medium of the *Bacillus* can equally condition its toxicity. With *S. damnosum* larvae the LC 50 of preparations can vary from 0.35 to 5 mg/l/ 10 mn (about 15 times) due only to the changes in the growth medium. The toxicity varies finally on the type of primary products used, the freeze dried powders being always more toxic than the air dried ones for example.

II. II. Blackfly larvae and their feeding behaviour :

The feeding modalities of blackfly larvae have been extensively studied. Concerning the ingestion of *B.t.* H14, it is important to remember that the larvae can ingest large particles (in the oder of 100 μ) as well as fine colloidal particles less than 1 μ. The fine particles ($\leq 2\,\mu$) which constitute an important part of the material ingested by the larvae (Wotton 1977) are trapped by an adhesive mucus which coats their filtrering organs (Ross and Craig, 1980) ; it consists essentially of passive filtration. The big particles are filtered by sieving action of the fan rays and ingested more or less rapidly according to their size and consistence. The intestinal transit time in *S. damnosum* is usually very short. In natural conditions a time of 2 to 15 mn is largely sufficient for most of the larvae.

II. III. Formulations :

There are generally two types of formulations, either the particles are big clumps or they are isolated spores and crystals. The efficacy of the former which are usually wettable powders, increases with increasing size of clumps up to an optimum (40 to 70 μ) varying from one formulation to another and according to the larval instar. The addition of a cement to aggregate spores and crystals decreases the efficacy considerably. The efficacy, in most cases, varies proportionnally with the exposure period, long exposures at low concentrations giving better results. By adding certain surfactants to primary powder aqueous suspensions one can noticeably increase or decrease their efficacy. The fine particle formulations which are generally water dispersible concentrates are better adapted to operationnal requirements. Their efficacy is usually not dependent on exposure time. The ingested mass of such fine particle formulations taken in by blackfly larvae is smaller compared with the big particle suspensions but their effectiveness can be as good because of a better contact crystals-digestive enzymes and a considerable increase in the number of active particles. This higher number of active particles moreover confers a better "carry" to this type of formulations than with the wettable powders where the number of particles is much more limited. Under natural conditions the turbidity of the water does not affect their efficacy contrary to formulations with big particles of which the efficacy decreases considerably with turbidity. It is very likely that in this case higher turbidity acts not by feeding inhibition but rather by increasing the speed of intestinal transit or by a competitive selection between *B.t.* H14 particles and natural particles uptake.

III. CONCLUSION

Considering the mode of nutrition of *S. damnosum* larvae, the hydrological conditions in West Africa and operationnal requirements, the most convenient *B.t.* H14 formulations for Onchocerciasis control are the water dispersible concentrates. The experience acquired with Teknar shows that the evaluation of this type of formulation is very similar to that of conventionnal insecticide formulations. The practical use of Teknar actually limits the use of *B.t.* H14 to dry season treatments in zones where the larvae are resistant to insecticides. However, the remarkable stability of the toxin in general and of Teknar in particular as well as the level of efficacy recorded with new experimental formulations offer very promising prospects.

Serious difficulties are usually encountered in the improvement of the formulations due to the generally poor relation between toxicity vis-à-vis mosquitoes and *Simulium* larvae. As long as *A. aegypti* biological titre is not a relevant criterian to estimate blackfly formulations efficacy, the chemical companies do not possess a bioassay procedure which can guide them in improving their formulations. They must send material to specialized laboratories with all the risks involved, which slows down the process of improving blackfly larvicide formulations.

We have noticed that a certain number of factors act separately or conjointly to determine the efficacy of *B.t.* H14 formulations. It is not less true that the toxicity of this entomopathogen to blackfly larvae is still a largely unexplored field where much more work needs to be done. It is to be recalled that *B. thuringiensis* H14 remains at present the only available alternative to the World Health Organization since the appearance of insecticide resistance in certains areas of the Onchocerciasis Control Programme which has been going on for six years with considerable success.

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SUMMARY

Since the appearance of resistance to insecticides in human onchocerciasis vectors in West Africa *Bacillus thuringiensis* H14 is actually the only alternative available to the Onchocerciasis Control Programme to continue its treatment. operations in the zones where resistance has been detected. The best formulations adapted to prevailing conditions in the Programme are the water dispersible concentrates. Among these, Teknar^{R} which has given the best results is commercially available and used operationnally. Its stability is such that drums can be stored in the field in the open sun for more than 16 months without any loss of activity. However, the dosages to be applied (1.6 mg/l/10 mn) and the relatively poor "carry" of this formulation limit its use to dry season treatments. Its use in rainy season (high water) is unrealistic from pratical and economical points of view. New formulations 2 to 4 times more effective have already been tested and should, when available, be possible to use pratically in the same conditions as the best chemical larvicides.

The effectiveness of blackfly larvicides is usually closely related to the formulation. With regard to *B.t.* H14 there is generally no relation from one formulation to another between the *A. aegypti* titre expressed in international units and the toxicity to blackfly larvae. The same applies with primary products when tested under natural conditions. In such conditions the time of intestinal transit in the larvae of *S. damnosum* complex is very short and a portion of the ingested crystals do not liberate their toxin in the gut. The more or less good digestibility of the crystal is a factor which limits the toxicity of *B.t.* H14 to blackfly larvae contrary to the larvae of mosquito which under bioassay conditions have a much longer intestinal transit time. Other factors condition, to varying degrees, the effectiveness of *B.t.* H14 formulations on blackfly larvae among which are : the growth medium, the size of clumps in the formulation, the presence or absence of surfactants, the exposure period and the turbidity of the river water. It is pratically actually impossible using bioassay on *A. aegypti* larvae to forecast the effectiveness of these formulations and even of the primary products on blackfly larvae. The chemical companies have to send their material to specialised laboratories which significantly slows down the process of improving their formulations.

The use of *B.t.* H14 in the O.C.P. has already reached an operationnal level. The rapid progress in the knowledge of this entomopathogen and the effort devoted by the firms into its further development will make it possible to fully integrate *B. thuringiensis* H14 in large scale vector control operations in developping countries as well as in developped countries.

Formulations	*A. aegypti* titre (I.T.U./mg)	CL 50 *S. damnosum* (mg/1/10 mn) (trough test)
TEKNAR(R) 402 I	540	0,24
TEKNAR 3 X	2150	0,05
TEKNAR H 28	≃ 1900	0,08
TEKNAR H 31	≃ 1900	0,078

Table I : Comparison between *A. aegypti* titre of Teknar formulations and their toxicity to *S. damnosum* complex larvae.

Formulations		*A. aegypti* titre (I.T.U./mg)	CL 50 *S. damnosum* (mg/1/10 mn) (trough test)
Wettable dispersible powder	W.D.P. 50 % (ROGER BELLON)	≃ 1500	0,18
	BACTIMOS(R) (SOLVAY)	6000	0,62
	VECTOBAC(R) 6108 II (ABBOTT)	2000	0,17
Water dispersible concentrate	TEKNAR 402 I	600	0,24
	TEKNAR H 28	≃ 1900	0,08
	R 1471 C.R. (SOLVAY)	1270	18

Table II : Comparison of various *B.t.* H14 formulations between *A. aegypti* titre and their toxicity to *S. damnosum* complex larvae.

Sample Strain	RB 1 WI.00	RB 2 WI.00	RB 5 1884	RB 6 1884	BTF 1136 WI 36-5	BTF 1137 WI 36-5
A. aegypti titre (I.T.U./mg)	6870	5470	8645	10720	7500	30000
S. damnosum titre (I.T.U./mg) (LAB. Test)	10142	10142	12814	17151	-	-
% mortality at 0.05 mg/1/10 mn (trough test)	94.9	69.4	84	40.3	85	91

Table III : Comparison of various freeze dried *B.t.* H14 powders between the *A. aegypti* titre and their toxicity to *S. damnosum* complex larvae both in the laboratory (24 h contact in distilled water) and in field

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