INTERNATIONAL SOCIETY ON TOXINOLOGY
EUROPEAN SECTION

PROCEEDINGS OF THE 6th EUROPEAN SYMPOSIUM

ON ANIMAL, PLANT AND MICROBIAL TOXINS.

Basle, 1984.

ENDOTOXIN OF *Bacillus thuringiensis* H14 :

MODE OF ACTION, FORMULATION AND APPLICATION AGAINST MOSQUITO AND BLACKFLY LARVAE.

(p. 21 - 31)

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Bacillus thuringiensis H14 was isolated in 1977 in Israel from a mosquito breeding pond (1). This isolate was subsequently described as serotype H14, variety israelensis in 1978 (2). The H14 serotype, pratically without activity against the larval stage of Lepidoptera was proved to be highly toxic for larvae of a wide range of mosquito (1, 2, 3, 4, 5; 6, 7, 8) and black fly species (9, 10, 11).

1. MODE OF ACTION.

Contrary to other serotypes, B.t. H14 produces crystals pleomorphic in shape and size (2). These crystals present a composite structure with several components or subunits differing in size and electron density that may be found separately or in a single inclusion and surrounded by an envelope (12). The larvicidal activity is exclusively associated with the toxin in the crystals and spores are not involved in the intoxication process (3, 13, 14). The δ-endotoxin is a high molecular weight protoxin which is, upon ingestion by susceptible larvae, very rapidely cleaved in smaller subunits in the presence of alkaline pH and suitable proteolytic enzymes in the gut (13, 15, 16, 17).
Very little is known on the mode of action of the toxin. Immediately after ingestion crystals are splitted and 30mn after most of them in the anterior stomach are dissolved and only empty envelopes can be found (18). The pathogenesis observed in mosquito larvae is relatively similar to that seen in Lepidoptera except that with the former death is extremely rapid (15 to 20mn with blackfly larvae treated at LC 100). The midgut epithelium is the main site affected by the toxin (19). Cells of gastric caeca and posterior stomach specialized in the absorbtion of amino acids and proteins are the most susceptible and are first affected. The first symptoms (cellular hypertrophy and epithelial lysis) are rapidely followed by a complete destruction of the midgut

epithelium (18, 20). It is generally accepted that the toxin of Lepidoptera active serotypes (20, 21, 22) as well as H14 serotype (23) interfers with the ability of larval midgut epithelium to regulate ion permeability. Pathogenesis in black fly larvae is simular to that observed in mosquito larvae (24).

2. PROSPECTS FOR USE UN VECTOR CONTROL.

B.t. H14 presents most of the characteristics required for use as a larvicide in the field of vector control :

- Toxicity : as already mentioned, B.t. H14 is highly toxic for a wide range of mosquito and black fly species. Among mosquitos, Aedes and Culex are more susceptible than Anopheles (this is probably due to the surface feeding habits of anopheline larvae). Different strains of a same species like A.aegypti may present significative differences in their susceptibility to B.t. H14 (7, 25). Variations have also been recorded between different Simulium species (26, 27). Young larvae of mosquito and black fly are 8 to 10 times more susceptible than late instar (11, 27, 28, 29, 30). The efficacy of B.t. H14 is not significantly affected by environmental conditions such as temperature, pH, salinity as long as they remain is their natural range of variations (31, 32, 33, 34). On the other hand, when these factors perturb the normal feeding behaviour of larvae they interfere with efficacy of B.t. H14 (27, 35, 36).

- Stability : the stability of the δ-endotoxin of B.t. H14 is well known, heat stability (1, 3, 4, 5, 37) and storage stability of powders (38, 39, 40) as well as aqueous suspensions (31, 41). Limited data concerning stability of commercial formulations are available. In one study (42) the half-lifes of 3 formulations : I water dispersible concentrate (W.D.C.) and 2 vettable powders, were respectively 18, 7 and 6 days at 50° C and it was concluded that B.t. H14 formulations are much less stable than the kurstaki formulations. The stability of the same above mentioned W.D.C. was tested in Ivory Coast and it was proved that drums can be stored in the field more than 16 months right in the sun without any loss of efficacy against black fly larvae (43). Actually drums are routinely stored several months in the sun in the Onchocerciasis Control Programme (OCP) in the Volta region area in West Africa and remain fully effective.

- Selectivity : extensive studies have been carried out in the laboratory (mammal safety testing) as well as in the field (environmental safety). They clearly indicate that B.t. H14 is non toxic and non pathogenic to mammals and poses no significant health hazard. It appears also innocuous to nearly all non target organisms by direct challenge in mosquito as well as in black fly larvae breeding environment. Recently it was shown that hydrolized toxin is highly toxic for mice upon injection (44). It is not known wether or not the fraction (polypeptide) active against diptera upon ingestion and mammals upon injection are the same. However this information has no practical incidence on the use of B.t. H14 in vector control operations.

Prospects for the development of a resistance : no signficative difference in the response to B.t. H14 occurs between insecticide- susceptible and - resistant strains of mosquitos whatever detoxification process involved (dehydrochlorinases, oxidases, esterases, GSH-transferases) (45). The susceptibility of the S.damnosum complex larvae to B.t. H14 is not affected by resistance to organophosphorous compounds (46, 47). The potential for the development of a resistance has been tested using Culex quinquefasciatus larvae. A total of 36 generations have been completed to date under selection pressure. A slightly increased tolerance to B.t. H14 was noted following the ninth generation but larvae recover normal susceptibility immediately after selection pressure have stopped (48). Conclusions allow for optimism regarding the effective use life of B.t. H14.

Mass production : a very rapid advance has been noted in the development of mass production of B.t. H14 adapted from techniques used in the production of other serotypes. In 1982, 600,000l of formulation have been produced for use in the OCP in West Africa. The quality of 32 batches (about 10,000 l each) was controlled through bioassay with both A.aegypti and Simulium larvae and was proved to be very satisfactory and constant (49).

3. DEVELOPMENT OF FORMULATIONS AND FIELD APPLICATION.

B.t. H14 must be properly formulated to be used against mosquito and black fly larvae. The main role of the formulation is to present the toxin in particles readily ingestible by larvae, to allow spraying and then to maintain the particles in the larval feeding zone. Formulations are obtained from primary ferment material either in liquid form by direct concentration (water dispersible concentrate, flowable) or spray-dried powders (wettable powders, granules, slow-release briquettes). Problems involved in the development of suitable formulations vary significantly according to the target and requirement for operational spraying.

3.1. Mosquitos.

First series of laboratory experimentations have clearly demontrated that the activity of B.t. H14 preparations against mosquito larvae was not related to the concentration expressed in weight/volume (e.g. mg/l) as usual with conventional insecticides but in weight/surface (e.g. g/m^2 or Kg/Ha). This is due to the insolubility of the toxin in water and in most of conventional solvents. For the same reason, activity is negatively correlated with larval density (32, 50, 51). Activity is also highly dependant on water turbidity and nature of the substratum in breeding sites or containers. When used in turbid water, B.t. H14 looses most of its activity. That can be explained by a trophic competition by larvae between natural matters and B.t. H14 particles or binding of crystals with natural particles which accelerates settling rate. When crystals have sunk, they are no longer available to kill larvae, either they are degraded by microorganisms or more simply covered by sediment and out of the larval feeding zone (32, 34, 52, 53).
These observations explain most of the results obtained in the field. B.t. H14 has been tested in a very wide range of mosquito habitats : irrigated pastures (28, 34, 55, 56), rice fields (57, 58, 59, 60, 61, 62), brackish water ans salt marshes (33, 63, 64, 65, 66, 67), logoons (68), wood land and snow pools (69, 70), tree holes (6, 71), tyres and containers (6, 71, 72, 73), and polluted environment : dairy lagoons, storm drains, sceptic tanks, cess pits, drains (34, 54, 56, 57, 63, 66, 74, 75, 76, 77, 78, 79). Dosages to apply vary from 0.2 to 10 Kg/Ha according to the nature of breeding site and the formulation used. The residual activity of conventional formulations (wettable powders or liquid suspensions) is very short (1 to 3 days) whatever dosages applied. Usually, treatments must be repeted weekly. In polluted environment, dosages must be increased 2 to 4 times and a tremendous increase

in dosages (e.g. 200 times) do not extend significantly residual activity. B.t. H14 has some persistance in clear water breeding sites without sediment like tyres and small containers.
With recently improved formulations it is possible to achieve a complete control, at least in clear water habitats, using no more than 0.1 Kg/Ha (80). Breeding sites covered by a plant canopy remains difficult to treat with conventional formulations. They can be treated from now with new granules formulations wich give satisfactory results with 5 to 10 Kg/Ha. Adsorption of B.t. H14 powder on a substrate like sand provides excellent results in polluted environment with only 0.15 Kg/Ha (81). New slow release briquettes are presently under field evaluation. With a rate of I briquette / 70-80 M^2 a good control could be achieved with a significant increase in residual activity. Briquettes could also be very usefull to control containers-breeding mosquitos (82).
From a general point of view, the improvment of B.t. H14 larvicides for mosquito control relates principally to the physical characteristics of formulations. From most of laboratory bioassays it appears that field efficacy of formulations is not correlated with potency in A.aegypti I.U./mg determined by standard titration in the laboratory. It is common to find a 200 I.U./mg formulation more effective in the field than a 5,000 I.U./mg one. The production of very high potency primary products is now feasible and will probably represent a non negligible part of the improvment of formulations. But firstly physical characteristics of formulations must be improved according to the feeding biology of the target and particularities of its breeding environment. At that time dosages in I.U./Ha could be significantly reduced, residual activity extended and then B.t. H14 could be used in a really cost effective manner.

3.2. Black flies.

Contrary to mosquitos, black fly larvae are breeding in fast runing water. They stay firmly attached on supports and collect food by filtration of suspended particles drifting in the river water by means of specialized cephalic fans. Larvae indistinctly ingest all kind of particles in a range from 0.1 μ to 200 μ approximativly. Big particles are trapped in cephalic fans by direct interception (active filtration). Ingestion

depends on size and concistence of particles and on the larval stage. Fine particles (0.1 to 5 µ) are trapped by an other mechanism. They are sticked on a muco-substance which coats the inner part of cephalic fans (passive filtration) and are more or less retained according to their physico-chemical properties like electro-static charge.
The only practical way to control black flies, almost in tropical area, is to suppress larval populations. Suitable larvicides are poured in the rivers immediately upstream the larval breeding sites. Contact between larvae and formulation is usually very brief. The efficacy of a black fly larvicide is estimated by the dosage itself (usually in mg/l applied to the discharge of the river during 10mn : mg/l/10mn) and the carry, a very important parameter, which is the distance downstream the treatment point along which the larvicide remains fully effective. Carry is usually dependant on the discharge of the treated river but a great deal also on the formulation itself. A formulation may present a satisfactory level of efficacy but a very short carry whatever discharge rate.
First laboratory bioassays and field trials have demonstrated the high level of toxicity of B.t. H14 for black fly larvae (9, 10, 11, 26, 83). Later on formulations have been field tested in temperate (84, 85, 86, 87) as well as in tropical (46, 47, 88, 89) areas.
In 1980 a strong resistance to temephos (Abate^{R}), an organo-phosphorous compound, have been recorded in 2 forests species of the S.damnosum complex, vectors of onchocerciasis, in the OCP area (90). This resistance was rapidely followed by a resistance to chlorphoxim, an other O.P. compound (91), and finally a cross resistance to most of the O.P. compounds usable in public health. In 1982 B.t. H14 was the only alternative for the continuation of larviciding in the zones where resistance has occured. A water dispersible concentrate is used at a dosage of 1.6 mg/l/10mn. Its carry during rainy season is limited in practice to 8-10 km. Even using very big helicopters for spraying, it is impossible to treat routinely rivers with discharge higher than 50 M^3/s. In addition the high viscosity of the formulation much complicates spraying operations. Consequently researchs have been carried out in order to improve formulations.

The first experimentations clearly demonstrated that the efficacy of formulations is much more dependant on the formulation itself that on the active ingredient (toxin) content in A.aegypti I.U./mg (27, 92, 93). Taking into account the important role of the formulation, studies have been carried out ot determine the best type of formulation to develop. There are generally 2 types of formulations : either the particles are big aggregates of B.t. H14 (mean size 10 to 30 µ) or they are suspensions of dispersed 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 the other and according to larval instar. The addition of a cement to aggregate spores and crystals decreases the efficacy considerably. This efficacy, in most cases, varies proportionally with the exposure period, long exposure at low concentrations giving better results. Under natural conditions these formulations decrease the efficacy significantly with water turbidity by a competitive selection between B.t. H14 particles and the uptake of natural particles by blackfly larvae (47). Consequently these formulations are much less effective during the rainy season than during the dry season with the lower turbidity in the rivers. On the contrary, the efficacy of formulations with isolated spores and crystals remains the same whatever turbidity in the rivers. Their efficacy is not dependant on the exposure time (a one minute exposure is sufficient) and finaly the high number of active particles (crystals) confers to this type of formulations a better carry than with the previous one in which the number of particles is much lower. These observations as well as operational requirements have demonstrated that the best type of formulation to develop for use (aerial spraying) in onchocerciasis control are liquid suspensions of dispersed crystals. Improvment of such formulations relates mainly to the increase in endo-toxin content of self-dispersive preparations.

A screening programme has been developed in West Africa (Ivory Coast) for the evaluation of experimental formulations. Presently more than 200 formulations produced by 4 companies have been tested under simulated field conditions. 6 of them have reached the stage of field evaluation. At present 2 formulations only have reached the stage of operational use. From recent bioassays and field evaluations it appears that in the near future, with normal or asporogenic strain, it should be possible to obtain B.t. H14 formulations giving 100% mortality at 0.8 mg/l/10mn with suitable physical characteristics. Nevertheless the probability is low, even with improved formulations, to achieve a satisfactory carry (e.g. 20 Km or more). On that point, B.t. H14 probably will never be competitive with good formulations of chemical insecticides like Abate and Chlorphoxim of which the carry is 40 to 60 km during rainy season (high discharge rate in the rivers). On the contrary B.t. H14 will be competitive for the dry season treatments (low discharges) where carry is always very limited whatever the insecticide and the formulation used.

CONCLUSION.

The development of B.t. H14 for mosquito and black fly control has been extremely rapid and probably sometimes too much. This bio-control agent presents several very interesting characteristics but its use must be limited to situations where it is or it will be cost-effective and when it provides significant benefits in terms of environmental safety and resistance to insecticides
In the field of mosquito control it was noted that potency of primary unformulated products was satisfatory but they must be properly formulated in order to reduce applied dosages and to increase the residual efficacy. B.t. H14 still remains relatively expensive compared with chemical larvicides. In addition to situations where environment protection is a priority, its use is undoubtly profitable in rotational use with chemical insecticides in mosquito control programmes. Thus it could provide a significant decrease in the selection pressure by chemicals and delay the appearance of resistance. B.t. H14 has been sucessfully used in a very wide range of mosquito habits but one must keep in mind that, if it is a very attractive bio-control agent, it cannot be used in situations where larviciding with chemical insecticides is not feasible.

B.t. H14 was early introduced in operational black fly control immediately after resistance to insecticides has occured. Researchs to find out the best type of formulation and then to improve it have started jointly. 2 years latter progress relates to a doubled level of efficacy of formulations and improvment in their physical characteristics. The use of an asporogenic strain do not present practical significant advantages exept when drinking water must be treated. But a such strain seems to produce high potency products, and could replace in the near futur the normal strains.
The use of B.t. H14 in Onchocerciasis Control Programme in West Africa proves that this agent can be routinely used in a very large scale vector control operation in tropical area. Up to the present its use in an emergency situation due to resistance to insecticides was very costly. This situation will change when the new improved formulations will be commercially available. At that time the use of B.t. H14 could be restricted to the dry season treatments where it is competitive with chemical larvicides. In return it should be extended to the treatment of most of the OCP area. This treatment scheme should represent, as already mentioned, a significant decrease in the process of selection pressure by chemical insecticides as well as in the pressure on environment in very sensitive ecological situations.

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