EFFECTS OF THE BACTERIAL MOSQUITO LARVICIDE, *BACILLUS THURINGIENSIS* SEROTYPE H-14 ON SELECTED AQUATIC ORGANISMS

TAKESHI MIURA, R. M. TAKAHASHI AND F. S. MULLIGAN, III

University of California, Mosquito Research Laboratory, 5544 Air Terminal Drive, Fresno, California 93727

ABSTRACT. *Bacillus thuringiensis* serotype H-14 deBarjac, at rates used for mosquito control, is very safe to organisms associated with mosquito breeding habitats. A total of 28 species or species groups was treated with the bacterium under simulated or field conditions; no adverse effects were noticed on the organisms, except chironomid larvae. Chironomid larvae were slightly affected. However, the effect was so light that the population in the field continuously increased after the treatment.

The rapid increase in mosquito resistance to various insecticides and growing public concern over environmental health have accelerated the search for other means of mosquito control such as use of biological agents or insect growth inhibitors. This laboratory, therefore, evaluated the larvicidal activity of bacterial agents in the laboratory and field against *Aedes* and *Culex* mosquitoes (Mulligan et al. 1978, 1980). Concurrently, studies were initiated to investigate the impact of bacterial agents on nontarget organisms.

This paper reports the effects of the use of *Bacillus thuringiensis* serotype H-14 deBarjac (*B. t.* H-14) on some common aquatic organisms found in mosquito breeding habitats in the San Joaquin Valley of California.

MATERIALS AND METHODS

B. t. H-14 used in this study was produced and formulated by Sandoz Inc. as a flowable formulation (SAN 402 I WDC) and it contained ca. 1.5×10^{10} viable spores/ml.

A simulated field test against crustaceans was conducted outdoors in 5-gal aquaria. A mixture of *Ceriodaphnia* sp., *Simocephalus* sp., *Moina* sp., Chydorinae, *Cyclops* sp., *Cyprois* sp. and *Hyalella azteca* (Saussure) was maintained in the aquaria containing 16 liters of water each; one aquarium was treated at a rate of 5.4×10^3 spore/ml of the bacterium and one was left as treatment check. Pre- and posttreatment population counts were made periodically for 27 days using a method described previously (Miura and Takahashi 1973) except as noted otherwise.

The field tests were conducted on 0.02 ha plots in the Tracy experimental plot. This experimental plot has been used extensively for evaluation of new promising candidate mosquito larvicides and their impact on nontarget organisms (Schaefer et al. 1976, 1978, Miura and Takahashi 1976, Miura et al. 1978). It is located near the south end of Jerry slough, northwest of Bakersfield, CA and surrounded by patches of irrigated farmland and undeveloped land. Natural populations of *Aedes nigromaculis* (Ludlow), *Ae. melanimon* Dyar, *Culex tarsalis* Coquillett and aquatic invertebrates were well established in the plot.

On August 16, 1979, flowable formulation of *B. t.* H-14 was applied to plot no. 11 at a rate of 0.25 kg/ha (ca. 1.3×10^3 spores/ml) with a hand sprayer to an *Aedes* mosquito population. On August 23, 1979, the plot no. 9 was treated with the same formulation at a rate of 1 kg/ha (ca. 5.4×10^3 spores/ml) to a *Cx. tarsalis* population.

Population census of zooplanktons and immature aquatic insects was taken by dipping with a long handled dipper (450 ml) from 10 semi-fixed stations along the bank, and the samples were transported

to the laboratory where population changes were monitored daily (Miura and Takahashi 1975).

Counts to measure fluctuations in abundance of free-moving aquatic insects in the treated plots were made daily using modified minnow traps (Miura and Takahashi 1975).

RESULTS AND DISCUSSION

A variety of aquatic nontarget organisms including crustaceans and insects was collected from the plots during the study period but only the most frequently collected ones are listed as follows: 3 genera of cladocerans, *Ceriodaphnia* spp., *Simocephalus* spp., *Moina* spp.; 1 genus of ostracods, *Cyprois* spp.; 1 species of copepods, *Cyclops vernalis* Fischer; 1 genus of clam shrimp; *Eulimnadia* spp.; 1 genus of mayfly, *Callibaetis* spp.; 1 genus of dragonflies, *Pantala* spp.; 1 genus of damselflies, *Enallagma* spp.; 1 genus of corixids, *Corisella* spp.; 2 species of notonectids, *Notonecta unifasciata* Guerin, *Buenoa scimitra* Bare; 6 species and 1 genus of dytiscid beetles, *Laccophilus maculosus decipiens* LeConte, *L. mexicanus mexicanus* Aubé, *L. m. atristernalis* Crotch, *Thermonectus basillaris* (Harris), *Rhantus gutticollis* (Say), *Copelatus chevrolati renovatus* Guignot, *Hygrotus* sp.; 3 species and 1 genus of hydrophilid beetles, *Hydrophilus triangularis* Say; *Tropisternus lateralis* (F.), *Berosus styliferus* Horn, *Helophorus* sp.; 2 species of chironomid midges, *Chironomus stigmaterus* Say, *Goeldichironomus holoprasinus* (Goeldi).

Results of the simulated field tests against crustaceans are shown in Fig. 1. There were no detectable effects of the bacterial treatment on the population fluctuation of the crustaceans tested in the aquaria.

Table 1 shows the data obtained from the field test against planktonic invertebrates. Only chironomid larvae collected immediately after treatment were affected; all larvae collected were killed within 2 days after the treatment. However, the counts of chironomid larvae in

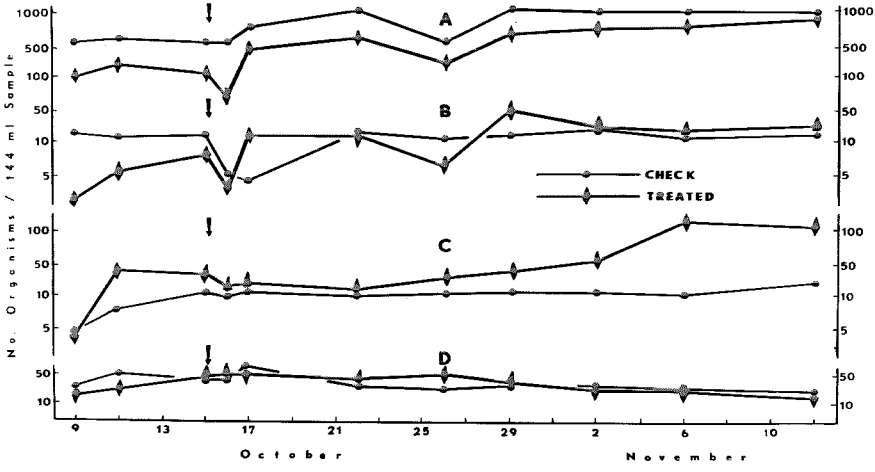

Fig. 1. Effects of *B. t.* H-14 (3.5 x 10^3 spores/ml) against zooplankton. A. Cladocerans; B. Side Swimmers; C. Ostracods; D. Copepods Arrows application days.

Table 1. Effects of *B. t.* H-14 (SAN 402 I WDC) on nontargets: Applied at 0.25 kg/ha against *Aedes* spp., Aug. 16, 1979.

Organism		Aug. 16	17	18	19	20
		No. Organisms in the Pre-Treatment water (4,500 ml) held in the Laboratory				
Cladocerans		7	14	14	14	14
Copepods		4	3	3	12	12
Clam Shrimp		4	4	4	4	4
Mayfly	N	17	17	17	17	17
Chironomids	L	8	8	8	8	8
		No. Organisms in water (4,500 ml) collected immediately after treatment and held in the Laboratory				
Cladocerans		12	12	12	22	26
Copepods		2	2	2	12	12
Clam Shrimp		3	3	3	3	3
Mayfly	N	12	12	12	12	12
Chironomids	L	7	4	0	0	0
		No. Organisms in water (4,500 ml) from Daily Collections				
Cladocerans		10	12	115	155	182
Copepods		3	9	26	23	36
Clam Shrimp		3	4	6	6	4
Mayfly	N	15	58	266	332	360
Chironomids	L	8	6	32	180	220
Beetle	L[a]	0	2	1	0	0

[a] = *Copelatus* spp.
L = Larvae.
N = Nymphs.

the daily collections from the treated plot increased rapidly indicating a short-term effect of the bacterial treatment and continuous reinfestation of the chironomid from the surrounding area. Garcia and Goldberg (1978) have reported that only a high concn (1×10^7 cells/ml) of bacterial treatment did have effects on chironomid larvae. Sinégre et al. (1979, Unpublished WHO Document) reported that the LC_{50} for chironomid larvae (*Chironomus* sp.) was ca. 0.1 mg/liter.

No adverse effects were noticed on other organisms found in the treated plot; even extremely chemically sensitive taxa such as cladocerans, clam shrimp and mayfly nymphs (Miura and Takahashi 1975, 1976) were unaffected.

The bacterium was not pathogenic to corixids, notonectids, hydrophilids (mostly *T. lateralis* and *H. triangularis*) and dytiscids (*T. basillaris* and *Laccophilus* spp.) (Table 2).

Table 2. Effects of *B. t.* H-14 (SAN 402 I WDC) on nontarget insects; applied at 0.25 kg/ha against *Aedes* spp., Aug. 16, 1979 (F. T. No. 79-6).

Insect	check			0.25 kg/ha		
	Aug. 17	18	19	17	18	19
Corixids	0	1	3	2	0	2
Notonectids	2	4	4	3	3	2
Hydrophilids	3	3	5	4	3	4
Dytiscids	35	59	26	24	50	36

Table 3. Effects of *B. t.* H-14 (SAN 402 I WDC) on nontarget; applied at 1.0 kg/ha against *Cx. tarsalis*, Aug. 23, 1979.

Organism		Aug. 23	24	25	26	27
		No. Organisms in the Pre-Treatment water (4,500 ml) held in the Laboratory				
Cladocerans		6	6	9	9	12
Copepods		29	36	61	88	101
Mayfly	N	47	47	47	47	47
Chironomids	L	4	4	4	4	4
Beetle	L	4	4	4	4	4
		No. Organisms in water (4,500 ml) collected immediately after treatment and held in the Laboratory				
Cladocerans		7	7	7	17	25
Copepods		18	71	83	102	173
Mayfly	N	64	64	64	64	64
Chironomids	L	13	1	0	0	0
Beetle	L	2	2	2	2	2
		No. Organisms in water (4,500 ml) from daily collection				
Cladocerans		7	36	55	22	—
Copepods		24	366	237	287	—
Clam Shrimp		0	3	0	0	—
Mayfly	N	55	606	544	468	—
Chironomids	L	9	30	63	34	—
Beetle	L	3	6	2	8	—

— = Dry.
L = Larvae.
N = Nymphs.

On August 23, a 4-fold concn of the bacterial spores was used to treat the plot but the effect due to the treatment was identical to those of the August 16 treatment (Table 3).

The data obtained from this study clearly indicate that this bacterium could be used safely without causing any damage to the major nontarget organisms associated with mosquito breeding habitats. Therefore, it can be utilized in a biological or integrated control program against mosquitoes.

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