EFFICACY OF TWO FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (H-14) AGAINST *AEDES VEXANS* AND SAFETY TO NON-TARGET MACROINVERTEBRATES[1]

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ABSTRACT. An experimental Sandoz formulation of *Bacillus thuringiensis* var. *israelensis* (SAN 402 SC 98) was several times more effective than Abbott ABG 6188 against larvae of *Aedes aegypti* and *Ae. vexans* in the laboratory. Field applications of SAN 402 SC 98 at 0.25 liter/ha and ABG 6188 at 1.00 liter/ha resulted in more than 97% control of *Ae. vexans* larvae after 48 hours, with residual activity of 24 hours or less. The amphipod, *Hyallela azteca*, and 4 species of water beetles were apparently unaffected by 100 ppm SAN 402 SC 98, which is 1,449 times the 24 hour LC_{50} for third instar *Ae. vexans* larvae.

INTRODUCTION

Since its discovery by Goldberg and Margalit (1977), *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) has proved to be a potent control for mosquito larvae. Margalit and Dean (1985) reviewed our knowledge of *B.t.i.*, reporting on its effectiveness against black flies and mosquitoes, including 21 species of *Aedes*, and its safety to non-target organisms and humans. High larvicidal activity against *Ae. vexans* (Meigen) was reported by Ramoska et al. (1982) and Clarke and Rowley (1984), and effectiveness against other North American *Aedes* in field trials was reported by Fanara et al. (1984), Eldridge et al. (1985), and Jones and Lloyd (1985).

This study evaluates the effectiveness of an experimental liquid formulation of *B.t.i.* made by Sandoz (SAN 402 SC 98) against larvae of 3 species of *Aedes* in the laboratory and against larvae of *Ae. vexans* in the field, and compares it with an experimental formulation by Abbott Laboratories (ABG 6188). *Aedes vexans* was selected for field tests because it is the most important urban nuisance species in most areas of northern U.S.A. (Horsfall et al. 1973). The effect of SAN 402 SC 98 on non-target macroinvertebrates was also investigated.

MATERIALS AND METHODS

Liquid *B.t.i.* formulations SAN 402 SC 98 (Lot BTI-184-P2) from Zoecon Corporation (Sandoz) and ABG-6188 (Lot 87-038-BA) from Abbott Laboratories were tested. Activity of both formulations was evaluated in the laboratory against second and fourth instar larvae of *Aedes aegypti* (Linn.), and third instar larvae of *Ae. vexans*, while SAN 402 SC 98 alone was tested against fourth instar larvae of *Aedes triseriatus* (Say). *Aedes aegypti* (Rockerfeller strain) and *Ae. triseriatus* larvae were from laboratory colonies, while *Ae. vexans* larvae were collected from a nearby marsh.

We used bioassay procedures similar to those employed by Lacey and Singer (1982). Four 100 ml replicates of 6 to 8 serial concentrations of both formulations of *B.t.i.* in Madison city water were placed in plastic cups, and 20 larvae were added to each cup and to 4 cups of city water that served as a control. No food was added during the test period. Tests were conducted at 26 ± 2°C, and mortality was recorded after 24 and 48 hours. A probit analysis (Finney 1971) was used to evaluate results.

The first of 2 field trials was initiated August 20, 1987 in a recently flooded area at the west edge of the University of Wisconsin-Madison campus. The dense vegetation in the flooded area was predominantly reed canary grass (*Phalaris arundinacea* Linn.), cattails (*Typha latifolia* Linn.) and arrowhead (*Sagittaria* sp.). Four plots measuring 40–50 m^2 with water depths of 10–20 cm and containing numerous third and fourth instar *Ae. vexans* larvae were used for the test. Treatments of 2.0, 1.0 or 0.5 liter/ha of SAN 402 SC 98, the most effective formulation in laboratory tests, were applied to 3 plots; the fourth plot was a control. All plots were separated by untreated areas. Treatments were made with a backpack sprayer using enough water to cover each plot. Surface water temperatures at the time of application ranged from 24 to 35°C because of variable shading, and the pH was 6.9.

Efficacy of treatment was determined by the method of Fanara et al. (1984). Ten random samples of mosquito larvae were collected with a 350 ml dipper from each plot immediately before treatment, and 1, 2, 3 and 7 days after treatment. Samples from each plot were com-

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bined in the field, larvae were separated from debris in the laboratory, then killed and preserved with ethanol. Numbers of young larvae (first and second instars), old larvae (third and fourth instars), and pupae were counted in the laboratory. Percent reduction of larval populations was calculated using a formula by Mulla et al. (1971).

A second field trial was carried out September 22, 1987, after heavy rain reflooded much of the same area. Fourteen 2 m square plots (4 m^2) were used. They were 10–15 cm deep, and contained large numbers of third and early fourth instar *Ae. vexans* larvae. Because no movement of larvae into treated areas from adjacent untreated areas was observed in the first field test, smaller plots were used to maximize treatments. Plots were separated by unflooded areas or by erecting wooden barriers (2.5 m × 20 cm) between them. Two replicates of each treatment and untreated controls included ABG 6188 at 1.00, 0.50, and 0.25 liter/ha, and SAN 402 SC 98 at 0.50, 0.25, and 0.10 liter/ha, applied evenly over each plot with a sprinkling can. Surface water temperatures varied from 22 to 26°C at the time of application. To evaluate larval populations, 5 samples were collected from each plot 1 day before and 1 and 2 days after treatment by using a dipper as previously described. Additional samples were not collected because rapid drying significantly reduced water levels and plot size.

Immediately after each treatment was completed, and again after 24 hours, samples of water were collected from the surface of each plot and returned to the laboratory for assay against larvae collected from an untreated area. Ten larvae were placed in each of two 100 ml aliquots from each plot to provide 4 replicates of each treatment rate.

The effect of *B.t.i.* on some non-target macroinvertebrates collected from permanent ponds was also evaluated in the laboratory using the method of Sebastien and Brust (1981). After preliminary tests, invertebrates were exposed in plastic cups to 100 ml suspensions of SAN 402 SC 98 at 50 and 100 ppm and an untreated control. Each species was tested separately, with no more than 11 in any container.

RESULTS AND DISCUSSION

Results of laboratory tests are summarized in Table 1. Twenty-four hour LC_{50} values of SAN 402 SC 98 were 0.155 ppm and 0.096 ppm for fourth instar *Ae. triseriatus and Ae. aegypti* larvae, respectively, and 0.069 ppm for third instar *Ae. vexans* larvae. After identical periods of exposure, this material was 5 to 7 times more effective than ABG 6188 in killing similar larval instars of *Ae. aegypti* and *Ae. vexans*, with second instar larvae of *Ae. aegypti* being significantly more susceptible to both formulations than the fourth instar. Except for the test of ABG 6188 against fourth instar *Ae. aegypti*, exposure for 48 hours instead of 24 hours did not appreciably increase mortality. The susceptibility to *B.t.i.* of all 3 species of *Aedes* used in these tests, and the greater susceptibility of earlier instars, corroborates findings of Wraight et al. (1981, 1987). The LC_{50} values of *Ae. aegypti* and *Ae. triseriatus* for SAN 402 SC 98 are similar to those reported by Lacey and Singer (1982).

Table 1. Larvicidal activity of two formulations of *Bacillus thuringiensis* var. *israelensis* against three species of *Aedes* in the laboratory. There was no mortality in controls.

Formulation	Exposure (hours)	LC_{50} ppm	95% fiducial limits (ppm)	LC_{90} ppm
Second instar *Ae. aegypti* larvae (laboratory colony)				
SAN 402 SC 98	24	0.039	0.034–0.044	0.100
	48	0.035	0.031–0.039	0.092
ABG 6188	24	0.275	0.241–0.309	0.759
	48	0.221	0.192–0.250	0.627
Fourth instar *Ae. aegypti* larvae (laboratory colony)				
SAN 402 SC 98	24	0.096	0.091–0.101	0.184
	48	0.092	0.085–0.099	0.172
ABG 6188	24	0.698	0.621–0.775	1.890
	48	0.488	0.438–0.538	1.267
Third instar *Ae. vexans* larvae (field collected)				
SAN 402 SC 98	24	0.069	0.061–0.077	0.184
	48	0.061	0.053–0.069	0.164
ABG 6188	24	0.358	0.340–0.376	0.830
	48	0.319	0.305–0.333	0.670
Fourth instar *Ae. triseriatus* larvae (laboratory colony)				
SAN 402 SC 98	24	0.155	0.137–0.173	0.348
	48	0.141	0.125–0.157	0.307

In the first field trial, 0.5 liter/ha or more of SAN 402 SC 98 produced 100% mortality of *Ae. vexans* larvae within 24 hours (Table 2). Three days after treatment and 8 days after flooding some first instar larvae appeared. A light rain 2 days after treatment, which caused no additional flooding, may have stimulated this hatch (Horsfall et al. 1973). Clarke and Rowley (1984), who achieved 100% control of *Ae. vexans* larvae 24 hours after treatment with *B.t.i.* at 1.0 liter/ha of Teknar® (Sandoz), also reported minimal hatching of additional eggs in the absence of significant rainfall.

Results of the second field trial are summarized in Table 3. As in laboratory tests, SAN 402 SC 98 was much more effective than ABG 6188, with more than 97% control achieved after 48 hours at 0.50 and 0.25 liter/ha., while ABG 6188 achieved 97% control only at 1.00 liter/ha. The 0.50 liter/ha SAN 402 SC 98 did not pro-

Table 2. Mean number of *Aedes vexans* larvae or pupae per dip and percent larval control (in parentheses) in 40–50 m^2 plots in flooded marshland treated with *Bacillus thuringiensis* var. *israelensis* (SAN 402 SC 98) August 20, 1987.

Treatment rate (liters/ha)	Larval instar	Pre-treatment	Days posttreatment			
			1	2	3	7
2.0	1 & 2	0.6	0.0	0.0	0.0	1.4
	3 & 4	26.8	0.0	0.0	0.0	0.4
	pupae	0.0	0.0	0.0	0.0	0.0
			(100)	(100)	(100)	(75.1)
1.0	1 & 2	5.2	0.0	0.0	0.0	0.0
	3 & 4	18.1	0.0	0.0	0.0	0.0
	pupae	0.0	0.0	0.0	0.0	0.0
			(100)	(100)	(100)	(100)
0.5	1 & 2	0.0	0.0	0.0	1.8	1.3
	3 & 4	21.3	0.0	0.0	0.0	0.7
	pupae	0.0	0.0	0.0	0.0	0.0
			(100)	(100)	(81.6)	(64.4)
Control	1 & 2	0.4	0.0	0.0	2.8	3.6
	3 & 4	23.5	29.1	16.5	8.2	2.7
	pupae	0.0	0.0	14.1	22.1	2.6

Table 3. Mean number of third and fourth instar *Aedes vexans* larvae per dip and percent control (in parentheses) in 4 m^2 plots in a flooded marshland with 2 replicates of each concentration of 2 formulations of *Bacillus thuringiensis* var. *israelensis* September 22, 1987.

Formulation	Treatment rate (liters/ha)	1 day pre-treatment	Days posttreatment	
			1	2
SAN 402 SC 98	0.50	23.1	1.3 (94.1)	0.2 (99.0)
	0.25	23.3	2.0 (90.9)	0.5 (97.6)
	0.10	21.5	3.8 (81.3)	3.6 (81.0)
ABG 6188	1.00	16.0	1.0 (93.4)	0.4 (97.2)
	0.50	18.8	11.3 (36.5)	9.6 (42.0)
	0.25	16.4	12.2 (21.4)	11.2 (22.5)
Control	0.00	24.3	23.0	21.4

duce 100% control as in the first trial, which could have resulted from the different method of application, the smaller plots, or more rapidly declining water levels. Using Tecknar (SAN 402 WDC), Ramoska et al. (1982) achieved only a 48-91% reduction of *Ae. vexans* larvae at 0.5 liter/ha, indicating the greater potency of SAN 402 SC 98. Twenty-four hour laboratory exposures using combined third and early fourth instar *Ae. vexans* larvae resulted in 100% mortality in water collected immediately after treatment from areas treated with 0.50 and 0.25 liter/ha of SAN 402 SC 98 and 1.00 and 0.50 liter/ha of ABG 6188. Treatments with 0.10 liter/ha of SAN 402 SC 98 produced 97.5% mortality and 0.25 liter/ha of ABG 6188 resulted in 67.5% mortality. Water collected after 24 hours produced no mortality at any treatment rate, showing that there was no residual activity of *B.t.i.* in the surface water.

The short residual activity of *B.t.i.* was reviewed by Margalit and Dean (1985). SAN 402 SC 98 at 100 ppm (1,449 times the LC_{50} for third instar *Ae. vexans* larvae) caused no mortality after 5 days to 5 *Peltodytes edentulus* LeConte and 10 *Haliplus immaculicollis* Harris (Coleoptera: Haliplidae), 30 *Hydroporus undulatus* Say and 11 *Laccophilus maculosus* Say (Coleoptera: Dytiscidae), and 30 *Hyallela azteca* (Saussure) (Amphipoda: Talitridae). There was also no mortality at 50 ppm or in the controls. Miura et al. (1980) reported that, except for Chironomidae, 28 species groups of non-target organisms associated with mosquito breeding habitats showed no adverse effects from treatment with *B.t.i.* Garcia *et al.* (1980) and Sebastien and Brust (1981) also found that *B.t.i.* caused negligible mortality to non-target organisms.

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