THE INFLUENCE OF EXTENSIVE AQUATIC VEGETATIVE GROWTH ON THE LARVICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN REDUCING *SIMULIUM VITTATUM* (DIPTERA:SIMULIIDAE) LARVAE IN THEIR NATURAL HABITAT.[1]

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ABSTRACT. The presence of extensive aquatic vegetative growth (*Potomogeton crispus* and *P. pectinatus*) had little effect on reducing the larvicidal activity of *Bacillus thuringiensis* var. *israelensis (Bti)* against *Simulium vittatum*. Following a 35 min exposure to 3.10 ppm (98,800 spores/ml) suspension of *Bti*, the reduction in *S. vittatum* larvae over a 96-hr period ranged from 27 to 92% along the entire 312 m length of the test stream.

INTRODUCTION

Field trials were conducted in early May 1980 to evaluate the effectiveness of *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*) in reducing *Simulium vittatum* Zetterstedt larvae in a moderately sized effluent stream located at Holston Army Ammunition Plant (HAAP), Kingsport, TN (Frommer et al. 1981a). As reported, these field trials were conducted when essentially no aquatic vegetation existed and when larval and pupal attachments were confined to rubble in the streambed. These studies revealed that following a 35 min treatment exposure to a 3.10 ppm (124,000 spores/ml) suspension of *Bti*, a 25% maximum reduction in larvae was achieved. By increasing the exposure time to 70 min with a 1.55 ppm suspension of *Bti*, the percent reduction in larvae following treatment was significantly increased along the entire test stream from 50 to 70% after 24 hr, 35 to 80% after 48 hr, and 25 to 78% after 72 hr (Frommer et al. 1981a).

Failure to significantly reduce larval populations following a 35 min exposure to *Bti* was most likely a result of inadequate exposure time and/or the effects of nonrandom movement of spores in flowing water. Still, these findings clearly demonstrate that *Bti* can effectively reduce black fly larvae in their natural habitat when extensive aquatic vegetative growth is not present.

During midsummer to early fall the environmental conditions of the test stream changed with the dense growth of the aquatic weeds (*Potomogeton crispus* L. and *P. pectinatus* L.). These weeds not only served as additional larval attachment substrates but, in the author's view, could potentially alter, negatively or positively, the larvicidal activity of *Bti* through filtering, channeling, and/or delaying treatment suspensions.

The current study conducted in July 1980 at HAAP investigated the effects of extensive aquatic vegetative growth of *P. crispus* and *P. pectinatus* on the larvicidal activity of *Bti*.

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of proprietary names does not constitute endorsement.

METHODS AND MATERIALS

The *Bti* used was an experimentally formulated powder (Lot No. 6478-194) provided by Abbott Laboratories with an

International Toxic Units (ITU)/mg rating of 800–1200. This powder was composed of *Bti* carried in a finely ground particulate clay.

A 402 m stream section containing no sharp bends or deep streambed depressions was selected as the test area. It ranged from 3.0 to 3.6 m in width and from 20 to 50 cm in depth. A flow rate of 0.46 ± 0.14 m/sec and a flow volume of 23,900 liters/min were determined by use of a Gurley Pygmy Type Current Meter, model 625. This flow volume, which is an increase over the previous study (Frommer et al. 1981a), was a combination of water from the plant and a natural stream also containing *S. vittatum* which drained into the test area.

There were 6 larval sampling stations along the test stream: an upstream control station immediately above the point of treatment application; and 5 downstream, below the treatment point, at 37 m, 91 m, 152 m, 244 m and 312 m.

The use of tiles as artificial substrates was not possible due to excessive growth of *P. crispus* and *P. pectinatus*. In this particular environment, nearly all larval attachments were confined to the distal ends of these weeds. Therefore, estimates of larval population levels throughout the entire test area were made by placing 3 tape samples, similar to those used by Boobar and Barnett (1978), at each sample station. These were arranged randomly across the streambed and secured by staking.

Samplers consisted of 30 cm sections of 5 × 10 cm lumber with 4 strips of colored manila masking tape, each secured by clips, spaced evenly across the length of the board. Each tape, whose sticky side was doubled under to provide greater strength, measured 2.5 × 5.0 cm, giving a total exposed surface area per sampler of 1000 cm^2. Use of multiple samplers permitted effective statistical analysis and estimates of fluctuations in larval attachments at the sample stations. These fluctuations resulted from new larval hatches and from drifting larvae originating from upstream and within the treated area, both prior to and following treatment. Because of the tendency of black fly larvae to drift, the effect of *Bti* on a static population could not be measured readily. Therefore, the effect of *Bti* on larval attachment rates was the primary parameter measured in this experiment, i.e., larval recovery following treatment.

Twenty-four hr attachment counts of all larval instars were made on each tape at the 6 sampling stations, including the control, for 5 days prior to treatment. Larval counts were conducted by clipping and gently lifting the individual tapes from the stream. Once counted, tapes were wiped clean and reclipped to the sampler base. Preliminary observations demonstrated that larval counts at 24 hr intervals were adequate to estimate population variations, since larval counts on tapes remaining undisturbed for 48 hr and 72 hr were not significantly different from those after 24 hr. Tapes left undisturbed longer than 72 hr tended to accumulate silt and algae and were less suitable as larval attachment substrates. Tapes at the downstream sampling stations were not wiped clean of larvae on the day of treatment. On the day of treatment, larvae on only one of the tapes at each sample station were counted immediately prior to treatment to assure that no dramatic change in population level occurred during the 24 hr immediately preceding treatment. Posttreatment larval counts were conducted every 24 hr for 4 days following treatment, using the same procedures as with pretreatment counts. The number of dead larvae remaining attached to tapes would be most marked during the first 24 hr sampling following treatment. Subsequent counts at 48, 72 and 96 hr measured the attachment recovery rate of surviving larvae, larvae hatched within the test area since treatment, and new larval drift and hatch from outside the test area.

Environmental dynamics of the stream dictated that each sampling station could not be considered independently for establishing larval population levels for that area but, due to larval drift, was depen-

dent on those areas upstream for an undetermined portion of that population. Polynomial regression analysis (Hogben et al. 1971) was used in estimating percent reduction in larval attachments by fitting a polynomial to individual data points; each expressed the difference between pretreatment and posttreatment larval attachment values as a function of distance and treatment time. Pretreatment values for downstream sampling stations were estimates of larval attachments expected to occur without treatment for each day following treatment application. These values were based on attachment counts from downstream sample stations prior to treatment and from counts prior to and following treatment at the upstream control. The upstream control was used as an estimate of daily fluctuations in larval populations following treatment for the downstream sampling stations. Posttreatment values were calculated using larval attachment counts from only downstream sampling stations following treatment.

Cases in which the posttreatment values exceeded pretreatment values resulted in negative percents, that is, an increase in larval attachments downstream following treatment application was retained in the analysis to provide a true estimate of the variability of the results.

Twelve data points, i.e., 4 tapes per 3 samplers, per distance and time combination, were used to provide a precise estimate of the confidence bands about the estimated percent reduction values. In addition, polynomials with confidence bands were fitted to pretreatment and posttreatment data to provide a trend profile for larval attachment counts as functions of distance for each posttreatment day.

Treatment concentration and exposure time were selected from LC90 bioassay values for a 30 min exposure (Frommer et al. 1980a) in anticipation of future operational conditions. This exposure time was extended slightly to accommodate priming and purging of the dispensing apparatus during treatment application. Likewise, the field treatment concentration was increased approximately 2 times over laboratory values in order to achieve an equivalency in larval mortality.

On the day of the field trial, 2,592 gm of *Bti* were weighed out using a triple beam balance and mixed with water in a 3-speed Waring industrial blender. This mixture was then added to a 208 liter capacity Nalgene container and brought to a final volume (208 liter) with tap water. The bottom outlet of the Nalgene container was connected to an electrical, 3,450 rpm centrifugal fluid pump. Connected to the pump was a Brooks® rotometer with a 19 liter per min capacity. The rotometer was calibrated to dispense 6 liters per min, producing a 3.1 ppm stream concentrate of *Bti*, i.e., an estimated 98,800 spores/ml, for 35 min. The estimated spores/ml reported in this study are slightly less than the 124,000 spores/ml stated in earlier field trials (Frommer et al. 1981a) with the same 3.10 ppm weight/volume expression. This slight difference is well within the limits of normal spore plate count variation, though the *Bti* evaluated here is rated at twice the ITU/mg from that previously used. Previous laboratory findings concerning treatment preparations of *Bti* (Frommer et al. 1980b) were used in estimating spores/ml corresponding to weight/volume expressions (ppm). Though spores/ml may not be directly and consistently correlated to toxicity, i.e., spore crystals (Dulmage 1971), they can be used as a reference point in determining the distribution patterns of *Bti*. The outlet tubing of the rotometer was connected to a T-shaped dispenser approximately 120 cm across with a nozzle at each end. The T-shaped dispenser was secured to a board with the ends resting on the stream bank.

RESULTS AND DISCUSSION

The lower 95% fiducial limits are referenced to provide a more conservative estimate of the percent reduction in the number of larvae per tape as a function of

Table 1. Reduction in *Simulium vittatum* in a test stream following a 35 min exposure to 3.10 ppm *Bacillus thuringiensis* var. *israelensis*—24 to 96 hr posttreatment.

	Downstream sample stations	Posttreatment intervals											
		24 hr			48 hr			72 hr			96 hr		
		Estimated[4] mean	95% CL[5] Lower	95% CL[5] Upper	Estimated[4] mean	95% Cl[5] Lower	95% Cl[5] Upper	Estimated[4] mean	95% CL[5] Lower	95% CL[5] Upper	Estimated[4] mean	95% CL[5] Lower	95% CL[5] Mean
Percent reduction[1]	37 m	43.7	27.3	60.1	79.7	66.3	93.0	61.2	39.6	82.8	91.9	74.3	100.0
	91 m	84.8	71.6	97.9	90.92	80.1	100.0	87.2	69.8	100.0	97.0	82.9	100.0
	152 m	100.0	88.5	100.0	100.0	91.7	100.0	98.4	82.1	100.0	99.7	86.4	100.0
	244 m	93.2	77.6	100.0	99.0	86.3	100.0	92.6	71.9	100.0	91.8	75.0	100.0
	312 m	84.6	67.2	100.0	67.1	53.6	80.6	80.7	58.8	100.0	71.6	53.8	89.5
Pretreatment[2]	37 m	16.6	9.8	23.3	14.1	9.5	18.7	14.0	9.3	18.7	13.3	8.8	17.7
	91 m	13.9	8.5	99.4	10.4	6.7	14.2	10.2	6.4	13.9	9.6	6.0	13.2
	152 m	12.4	7.3	17.5	8.3	4.9	11.8	8.0	4.5	11.6	7.5	4.1	10.9
	244 m	10.5	4.0	17.0	6.5	2.1	10.9	6.3	1.8	10.7	5.6	1.4	9.9
	312 m	7.5	0.7	14.4	3.9	0.0	8.6	3.9	0.0	8.6	3.2	0.0	7.7
Posttreatment[3]	37 m	9.2	6.6	11.9	1.5	1.0	2.0	1.7	1.2	2.3	0.7	0.4	1.0
	91 m	2.4	0.3	4.6	0.7	0.3	1.1	0.6	0.2	1.1	0.3	0.8	0.5
	152 m	0.0	0.0	2.0	0.1	0.0	0.5	0.0	0.0	0.5	0.1	0.0	0.2
	244 m	1.0	0.0	3.5	0.0	0.0	0.5	0.1	0.0	0.6	0.0	0.0	0.2
	312 m	1.62	0.0	4.3	0.8	0.3	1.4	0.7	0.1	1.2	0.0	0.0	0.5

[1] Percent reduction values are based on polynomials fitted to individual data points; each expressed as the difference between pretreatment and posttreatment values for each time-distance combination.

[2] Pretreatment values are based on larval attachment counts from downstream sample stations prior to treatment application and the upstream control prior to and following treatment.

[3] Posttreatment values are based on larval attachment counts from downstream sample stations following treatment application.

[4] Estimated means for the number of larvae/tape for percent reduction, pretreatment and posttreatment values, each determined by polynomial regression analysis ($y = \beta_0 + \beta_1 D + \beta_2 D^2 + \beta_3 D^3$ where d = distance.

[5] 95% confidence limits.

time-distance. The fiducial limits for percent reductions were based on individual estimates of the standard error for each distance-time combination to take into account restriction of the outcomes near 100% and 0%.

As shown in Table 1, following the 35 min exposure to 3.10 ppm of *Bti*, the reduction of *S. vittatum* larvae along the entire 312 m length of the test stream ranged from 27 to 89% after 24 hr, 54 to 92% after 48 hr, 40 to 82% after 72 hr, and 54 to 86% after 96 hr. Clearly, this demonstrates that the presence of extensive aquatic vegetative growth had little effect on reducing the larvicidal activity of *Bti*. These results, as with others (Undeen and Berl 1979), suggest that laboratory bioassay data can be used, with some degree of confidence, in establishing dose-time criteria for field treatment application.

The present data are a sharp departure from earlier field trials where only a 25% reduction in larvae was achieved following a 35 min exposure to 3.10 ppm *Bti*. On the other hand, it is very similar in response to previous test results (25 to 80%) where the exposure time was increased to 70 min at 1.55 ppm *Bti* (Frommer et al. 1981a).

The reason for the sharp departure from the previous 35 min exposure results is not clear. A probable explanation is that *P. crispus* and *P. pectinatus* acted to retard the rate at which spores moved through the test area. The retardation of the treatment suspension can be seen clearly in the spore distribution patterns where delays in the anticipated arrival of the treatment suspension range from 2 to 6 min for each downstream sampling station to 152 m, and up to 11 min at 312 m. Such delays could have allowed more time for greater spore dispersion, even at 312 m where the treatment level dropped dramatically from 218 ppm to 0.8 ppm following a stable profile through 152 m (Frommer et al. 1981b). Another possible cause for this difference is that the *Bti* used in our previous field trial was rated at half (400–600) the ITU/mg as the one currently being evaluated. However, recent unpublished bioassay tests conducted at this laboratory using *S. vittatum* revealed there was little difference in the larvicidal activity between the 2 formulations.

The validity of comparing ITU/mg when working with simuliids is in question, since these units are established from bioassays using the 4th instar larvae of *Aedes aegypti* (Linn.) and not with black fly larvae.

Approximately 15% of larval reduction occurring during the first 24 hr post-treatment period consisted of early instars. Subsequent larval reattachments at 48, 72 and 96 hr had increases of 20, 25 and 30%, respectively. Assuming a constant rate of larval reattachment and development, complete recovery could have potentially occurred 15 to 22 days following initial treatment application. The increases in early instars were most likely, though not determined, from new egg hatches since treatment and from upstream drift.

A major factor which must be stressed is that concentration-time response is heavily dependent on environmental and physical conditions of the test stream at the time of treatment. If effective larval reductions are to be realized over known distances, then the above conditions must be known prior to treatment.

The set of environmental and physical circumstances encountered in this study indicates that a 100% larval reduction is not a realistic nor obtainable goal. Test data estimates support only a 27 to 92% reduction. Reasons for less than 100% reduction include the heavily infested larval population, constant drifting of all larval instars from untreated areas, and new hatches from within and upstream of the test area.

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