THE EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN REDUCING *SIMULIUM VITTATUM* (DIPTERA:SIMULIIDAE) LARVAE IN THEIR NATURAL HABITAT WITH NO EXTENSIVE AQUATIC VEGETATIVE GROWTH[1]

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ABSTRACT. A 35 min exposure of a 3.10 ppm (124,000 sp/ml) suspension of *Bacillus thuringiensis* var. *israelensis* (*Bti*) resulted in only a 25% reduction in *S. vittatum* larvae 24 hr following treatment 312 m downstream from treatment application. Increased exposure time to 70 min with 1.55 ppm (41,000 sp/mL) suspension of *Bti* resulted in a posttreatment reduction in the number of *S. vittatum* larvae along the entire 312 m test stream of 50–70% at 24 hr, 35–80% at 48 hr and 25–78% at 72 hr.

INTRODUCTION

Preliminary field trials with *Bacillus thuringiensis* var. *israelensis* (*Bti*) performed by this laboratory were of limited success in suppressing *Simulium vittatum* larvae. These studies demonstrated that a concentration of particulate material, when applied to a flowing stream for a defined period of time, spreads out as it is carried downstream resulting in a reduction in the intended dosage, in terms of concentration, but an increase in intended dosage in terms of time. Laboratory bioassay studies were later initiated to investigate the relationship between the concentration of *Bti*, the duration of exposure and larval mortality. These studies revealed that, with *S. vittatum* larvae, the LC_{50} and LC_{90} values of *Bti* decreased significantly as exposure time increased up to 60 min. Exposures longer than 60 min had little additional larvicidal effect (Frommer et al. 1980b). Our laboratory findings and others (Undeen and Berl 1979, Undeen and Nagel 1978), clearly demonstrated that *Bti* has high potential for use as a larvicide in the field. Recent field trials, conducted by Undeen and Colbo (1980), in several small streams in Newfoundland, confirmed the efficacy of *Bti* by producing up to 100% mortality of simuliid larvae.

The purpose of this study was to evaluate the potential of *Bti* for controlling *S. vittatum* larvae in their natural habitat. This was accomplished by exposing larvae to known concentrations of *Bti* for specific periods of time. A moderate sized stream located on Holston Army Ammunition Plant (HAAP) Kingsport, TN, was used as a test site. Selection of a moderate sized stream allows more realistic extrapolation to large-scale control operations.

The stream used as a test site was one of 5 effluent streams located at Holston AAP. Water in the test stream was drawn from the Holston River at a constant rate for use as a coolant in the manufacture of high explosives. The effluent streams by which the water returned to the river were of sufficient slope to provide a high level of agitation and were an ideal breeding environment for *S. vittatum*. Slight thermal pollution created by the plant appeared to exclude most benthic species except black flies. The thermal pollution provided a water temperature of 16° to 19°C throughout the year and probably contributed to black fly population stability. *S. vittatum* larvae and pupae constituted the great bulk of benthos and

[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.

covered nearly all available surfaces on rocks and aquatic vegetation during the spring, summer and fall. Black flies at Holston AAP consisted virtually of a pure population of *S. vittatum*. This species is apparently multivoltine with some adult emergence occurring on warm winter days. Overwintering most commonly is passed in the larval stage (Stone and Snoddy 1969).

The black fly population level remains relatively constant during the spring, summer, and fall, but the natural substrate for larval attachment changes during the summer as more aquatic vegetation emerges. During mid-summer and early fall, a dense growth of *Potamogeton crispus* and *P. pectinatus* occurs in the stream and serves as an additional attachment substrate for the larvae. Thus, our evaluation of *Bti* consisted of several parts. The first, reported here, was conduced in early May 1980, when essentially no aquatic vegetation existed and when larval and pupal attachment was confined to rubble in the stream bed. A later study will investigate the effects of extensive aquatic vegetation on the distribution and larvicidal capability of *Bti*.

METHODS AND MATERIALS

The *Bti* used was an experimental formulated powder (Lot No. 6406–125) provided by Abbott Laboratories containing 400–600 ITU/mg. This powder was composed of *Bti* carried in a finely ground particulate clay.

A 402 m stream section containing no sharp bends, or deep stream bed depressions, was selected as the test area. It ranged from 3.0 to 3.6 m in width and from 20 to 46 cm in depth. A flow rate of 0.55 m ± 0.12 m/sec and a flow volume of 18,269 liters/min was determined by use of a Gurley Pygmy Type Current Meter, model 625. This flow 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 sample stations along the test stream; an upstream control station immediately above the point of treatment application and 5 downstream stations at 37 m, 91 m, 152 m, 244 m, and 312 m below the treatment point.

Estimates of larval population levels throughout the entire test area were made by placing 15, 15 cm × 15 cm red unglazed fireplace tiles, similar to those used by Lewis and Bennett (1974) at each sample station. The tiles were arranged in 3 rows of 5 tiles each across the stream bed. The use of multiple tiles 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 readily be measured. Therefore, the effect of *Bti* on larval attachment rates was the primary measurement in this experiment.

Daily (24 hr) larval attachments counts (all instars) were made on each tile at all 6 sample stations, including the control, for 5 days prior to treatment. Each tile was removed from the stream bed, and larvae were counted on the upper tile surface. The tile was then wiped clean and returned to the stream bed. Preliminary observations demonstrated that larval counts at 24 hr intervals were adequate to estimate population variations, since larval counts on tiles remaining undisturbed for 48 hr and 72 hr were not significantly different from those after only 24 hr. Tiles left undistrubed longer than 72 hr tended to accumulate silt and algae and were less suitable as larval attachment substrates. Tiles at the downstream sample stations were not wiped clean of larvae (all instars) on the day of treatment. On the day of treatment larvae on only 1 of the tiles 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. Post

treatment larval counts were conducted every 24 hr for 3 days following treatment, using the same procedures as with pretreatment counts. Due to the rapid activity of *Bti*, and since little residue remains following treatment application (Frommer et al. 1981) observable mortality effects, that is, the number of dead larvae remaining attached to tiles, would be most marked during the first 24 hr following treatment. Subsequent counts at 48 and 72 hr measured the rate of attachment recovery of surviving larvae, i.e., larvae hatched since treatment from within the test area and new larval drift and hatch from outside the test area.

Environmental dynamics of the stream dictated that each sample station could not be considered independently for establishing larval population levels for that area, but, due to larval drift, was dependent on those areas upstream for an undetermined portion of that population. Since simple before and after treatment comparisons of larvae would not take into account the inherent dynamics of the effects of *Bti*, polynomial regression analysis (Hogben et al. 1971) was used in estimating percent reduction in larval attachments. This was accomplished by fitting a polynomial to individual data points, i.e., larval/tile/sample area, each expressing the difference between pre and posttreatment larval attachment values as a function of distance for both treatment times used. Pretreatment values for downstream sample 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 sample stations. Posttreatment values were calculated using larval attachment counts from only downstream sample stations following treatment.

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

Fifteen data points 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 pre and posttreatment data to provide a trend profile for larval attachment counts as functions of distance for each posttreatment day.

Treatment concentrations and exposure times were selected from LC_{90} bioassay values for 30 and 60 min exposures (Frommer et al, 1980b) in anticipation of future field operational conditions. These exposure times were slightly extended to 35 min and 70 min to accommodate priming and purging of the dispensing apparatus during treatment application. Likewise, field treatment concentrations were increased approximately 2 × over laboratory values in order to achieve an equivalency in larval mortality. In the event the 35 min treatment rendered ineffective results sufficient time would be allotted prior to conducting the 70 min treatment to allow for repopulation and to minimize, through acclimatization, any potential stress on surviving larvae caused from the effects of *Bti*.

On the day of each trial, 35 and 70 min applications, 2,000 gm of *Bti* were weighed out using a triple beam balance (2,610 gm capacity) and mixed with water in a 3-speed Waring® industrial blender. This mixture was then added to a Nalgene® 208 liter capacity container and brought to a final volume with tap water of 208 liter. 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 liter per min producing a 3.10 ppm stream concentrate of *Bti*, i.e., esti-

mated 124,000 spores/ml, for the 35 min exposure and 3 liter per min for a 1.55 ppm stream concentration of *Bti,* i.e., estimated 41,000 spores/ml, for the 70 min exposure. Previously, laboratory findings concerning treatment preparations of *Bti* (Frommer et al. 1980a) 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 referred to in order to provide a more conservative estimate of the percent reduction in the number of larvae per tile as a function of 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 Figure 1, the 35 min exposure to 3.10 ppm of *Bti* resulted in little overall reduction in larvae throughout the test area. Only at the 312 m sample station were the posttreatment larval counts lower than pretreatment indicating approximately a 25% reduction in the number of larvae per tile. Post-treatment larval counts at 37 m were higher than pretreatment, indicating a slight increase in the population at that point and time.

Failure to reduce larval counts through the 152 m sample station is difficult to explain, especially when the treatment concentration levels were relatively stable up to that sample point (Frommer et al. 1981). The most likely explanations are inadequate exposure time and/or the effects of nonrandom movement of spores in flowing water. Dukta and Kwan (1980) best characterized the phenomenon of spore distribution, indicating that bacteria do not form a complete homogeneous system with flowing water, but act as particulate matter precipitating to the sediment or are absorbed to larger floating particles or are caught in eddies, to be released later or resuspended into the main water flow to be carried farther downstream.

On the other hand, the marginal success in larval reduction at 312 m could be attributed to a more homogeneous dispersion of spores, even with the sudden drop from 2.5 ppm to 1.5 ppm in treatment concentration level at that point (Frommer et al. 1981). Nevertheless, laboratory bioassays that show a 1.5 ppm of *Bti* when exposed to larvae for 30 min, are within confidence limits of achieving 90% mortality (Frommer et al. 1980b).

Results from our data support the concept of nonrandom movement of spores in flowing water. However, the amount of variation was so small that statistical estimates of spore distribution and dissipation could accurately be established (Frommer et al. 1981).

Due to the minimal effects of *Bti* on larvae following the 35 min treatment, a delay of 3 days followed prior to conducting the 70 min exposure application.

Figures 2 to 4 illustrate the larvicidal activity had significantly increased when the exposure time was expanded to 70 min at 1.55 ppm of *Bti.* The percent re-

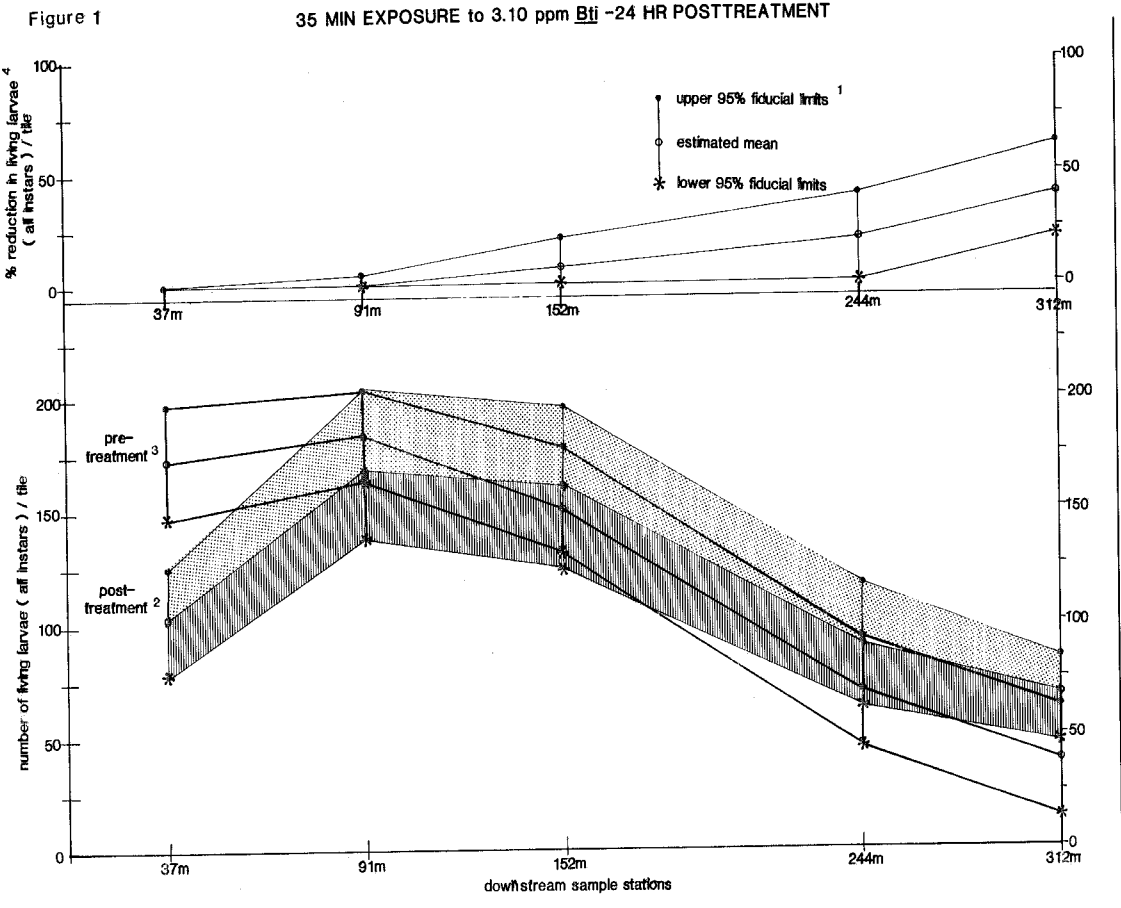
[1] Estimated mean number of larvae/title for per cent reduction and pre and posttreatment, each determined by polynomial regression analysis ($\hat{y} = \beta_0 + \beta_1 D + \beta_2 D^2 + \beta_3 D^3$ where D = distance)

[2] Pre treatment values are based on larval attachment counts from downstream sample stations prior to and following treatment.

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

[4] Percent reduction values are based on fitted polynomial to individual data points, each expressed as the difference between pre and posttreatment values, for each time-distance combination.

Figure 1



[1] See Figure 1, Footnote 1.

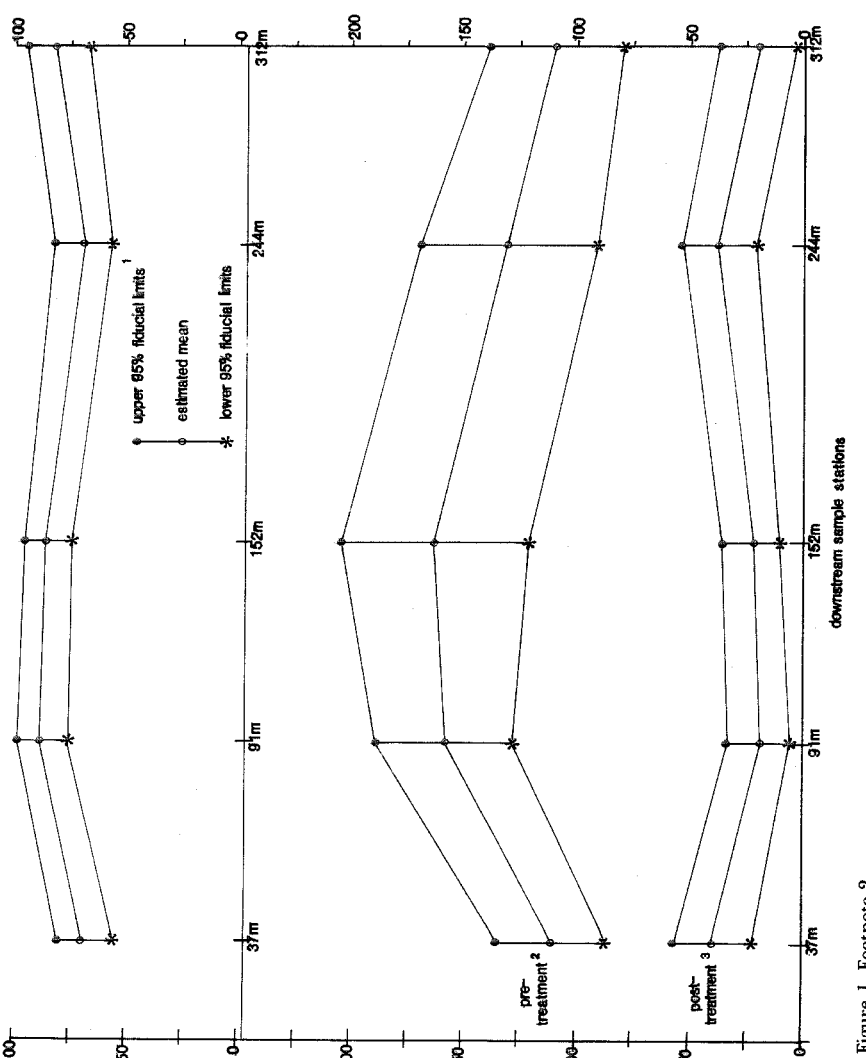


Figure 2

[2] See Figure 1, Footnote 2.

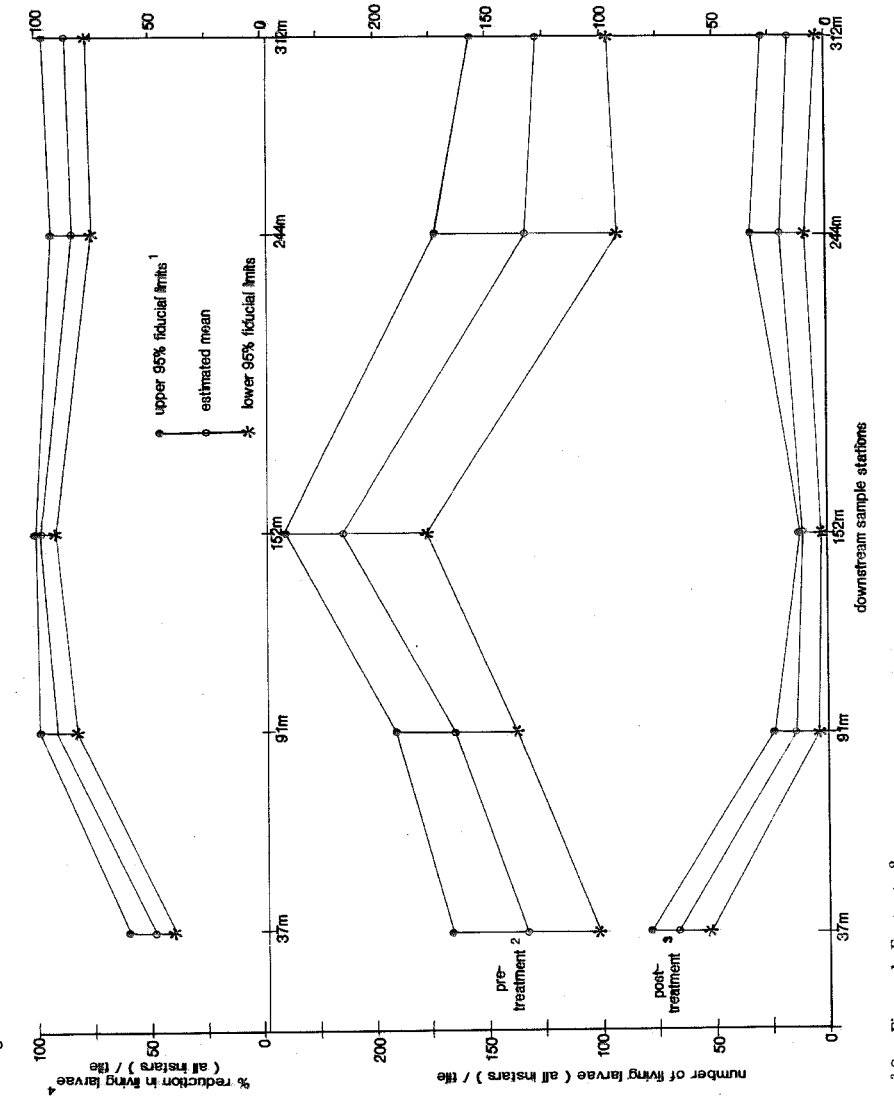


Figure 3

[3] See Figure 1, Footnote 3.

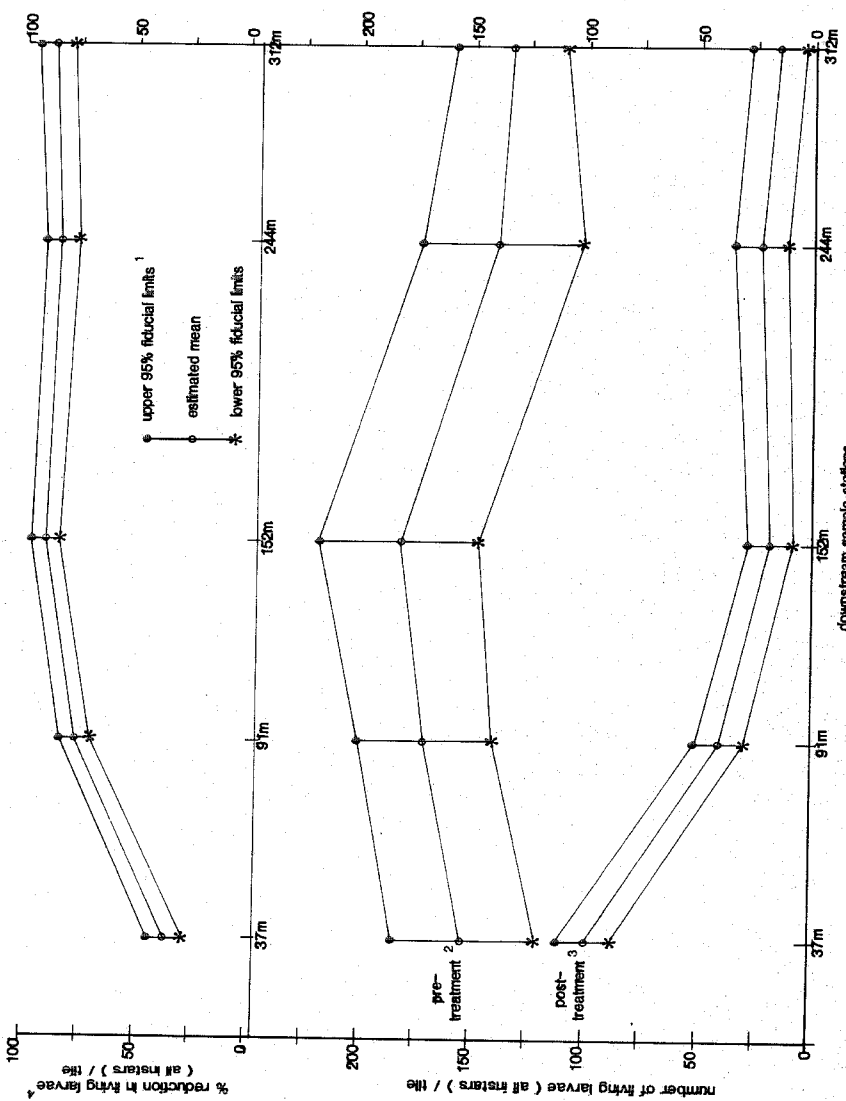


Figure 4

[4] See Figure 1, Footnote 4.

duction in the number of larvae (all instars) per tile following treatment ranged from 50 to 70% at 24 hr, 35 to 80% at 48 hr and 25 to 78% at 72 hr along the entire test area. It is apparent the increase application/exposure time not only led to greater spore dispersion, but increased spore contact with larvae.

Approximately 20% of the larvae attached to tiles following the first 24 hr posttreatment period were early instars. Subsequent larval reattachments at 48 and 72 hr had increases of 25 and 30%, respectively. Assuming a constant rate of larval reattachment and development, complete recovery could have potentially occurred 14 to 20 days after initial treatment. The increases in early instars were most likely, though not determined, from new egg hatches since treatment and from upstream drift.

It should be emphasized that complete (100%) larval reduction is not a realistic nor obtainable goal due to the constant drifting of larvae (all instars) in addition to new hatches from within and upstream of the test area. Our best estimates support only a 25 to 80% reduction, which is considerably more conservative than the 100% reported by Undeen and Colbo (1980).

Caution should be taken when comparing data due to the wide variation in environmental conditions and testing methodologies.

The results of these field trials in a moderate sized stream clearly demonstrate that a high degree of confidence can now be placed in *Bti*'s larvicidal capability for use in large-scale control operations.

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