THE DISTRIBUTION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN FLOWING WATER WITH NO EXTENSIVE AQUATIC VEGETATIVE GROWTH[1]

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ABSTRACT. Fifty to eighty percent (1.5 to 2.5 ppm) of a desired 3.10 ppm (124,000 sp/mL) treatment concentration of *Bacillus thuringiensis* var. *israelensis* (*Bti*) was sustained over a 20 to 22 min time interval. Peak recovery occurred midway through a 35 min exposure period when treating a 312 m section of stream. Little, if any, spore residual remained in the test stream following treatment application, as noted by the rapid decline in recovered spores once application was terminated and by the near background count levels of spores remaining in stream eddies 24 and 48 hr post-treatment.

INTRODUCTION

A critical factor in producing effective larval mortality in *Simulium vittatum* in the laboratory and field is the concentration-time exposure or the relationship between the concentration of the control agent used and the duration of exposure to that concentration (Frommer et al. 1980a and Undeen and Colbo 1980). In early May 1980, at Holston Army Ammunition Plant (HAAP), TN, a moderate size effluent stream was selected to study the effects of flowing water on the maintenance of certain concentration levels of a candidate microbial control agent, *Bacillus thuringiensis* var. *israelensis* (*Bti*), for specific periods of time over known distances.

METHODS AND MATERIALS

The *Bti* used was an experimentally formulated powder provided by Abbott Laboratories (Lot No. 6406–125) containing 400–600 ITU/mg. A 402 m section of the test stream with no extensive vegetative growth or major physical obstacles i.e., sharp bends, deep stream bed depressions, or constrictions in stream width was chosen as the test area. The test area ranged from 3.0 to 3.6 m in width and from 20 to 46 cm in depth. Flow rate was 0.55 ± 0.12 m/sec with a flow volume of 18,269 liters/min as determined with a Gurley Pygmy Type Current Meter, model 625. This flow was a combination of water from the plant and a natural stream that drained into the test area. Treatment consisted of producing a 3.10 ppm stream concentrate of *Bti*, i.e., an estimated 124,000 spores/ml, for 35 min. Estimated spores/mL were determined, using polynomial regression analysis, from laboratory data concerning concentration preparations (Frommer et al. 1980b). The concentration levels of spores passing through sampling stations at 37 m, 91 m, 152 m, and 312 m downstream from the treatment site were determined at various times after initiation of treatment.

It should be noted, though spores/ml may not be directly and consistently correlated to toxicity (Dulmage 1971), their use in discussing movement of treatment suspensions is valid. The intent of this study was not to examine toxicity patterns of spore crystals, but only to examine the distribution of *Bti* using spores/ml as a reference.

The sampling procedure described below for monitoring the distribution of

[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.

Bti was patterned after work in analyzing herbicide residues and dissipation in irrigation canals (Anderson et al. 1978, Demint, R. J. 1971, Frank et al. 1970).

A pretreatment water sample was collected from each sampling station. Further samples were collected at timed intervals as follows: (a) 37 m, 1st sample collected 2 min after initiation of treatment, and at 2 min intervals up to 12 min, and at 5 min intervals to 52 min followed by a final sample 10 min later; (b) 91 m, 1st sample collected 4 min after initiation of treatment and at 2 min intervals for the first 14 min, then at 5 min intervals up to 54 min and a final 64 min sample; (c) 152 m, 1st sample 6 min after initiation of treatment and at 2 min intervals for the first 16 min, then at 5 min intervals up to 56 min and a final 66 min sample; (d) 312 m, first sample collected 11 min after initiation of treatment and at 2 min intervals for the first 21 min, then at 5 min intervals up to 66 min. All 1st samples were collected, based on time-distance, and stream flow, when the leading edge of the *Bti* treatment would initially arrive at each downstream sample station.

Water samples were collected in Corning® 75 ml tissue culture flasks by submerging a single flask midstream at a medium water depth for each time-distance, then tilting at an approximate 45 degree angle, facing upstream, and allowing to fill with water. Water samples were kept refrigerated at 4°C until processed in the laboratory. On the day plate counts were prepared, a 10 ml aliquot of each sample was removed from the tissue culture flask after the flask was shaken vigorously by hand, and placed in a sterile Pyrex culture tube. Each 10 ml aliquot was heat shocked in a Tecan® water bath for 30 min at 60°C prior to preparation of the dilutions to be plated. Samples were heat shocked to eliminate background vegetative stage bacterial contaminants and nontoxins containing vegetative cells of *Bti* in the inoculum.

Sterile 9 ml distilled water blanks were used to prepare tenfold serial dilutions of the samples. Samples were plated undiluted where necessary. Dilutions were parapred using Falcon® plastic disposable 1 ml pipettes, then thoroughly mixed with a Lab Line Super Mixer®. One ml of each dilution to be plated was added to the 100 × 15 mm petri dish and placed on an Eberbach clinical rotator. Approximately 15 ml of Difco® tryptose blood agar base were added to each plate using a Brewer® automatic pipetting machine with sterile syringe and tube. Agar was maintained at 50°C throughout the procedure. Mixing of dilutions and agar was timed for 2 min followed by incubation for 24 hr at room temperature.

The number of colonies resulting were counted using a Fisher colony counter. The dilutions selected to determine the concentration (i.e., the number of colony forming units per ml (cfu/ml) were represented by the plate containing between 30 and 300 colony forming units.

Three plate replications from each collected field sample were then prepared for estimating mean spores/ml for each time and distance using polynomial regression analysis (Hogben et al. 1971). This statistical procedure provided an estimate of the spore suspension profile with respect to time as it moved through each of the downstream sample stations.

The correlation of the number of spores/ml recovered from the test area with that applied (wt/vol or ppm) was determined using previously acquired laboratory data on concentrations (Frommer et al. 1980b).

RESULTS AND DISCUSSION

Figures 1 to 4 illustrate the distribution and dissipation of spores as they move through 4 downstream collecting stations. All pretreatment background spore counts were at insignificant levels and thus did not interfere with the analysis of samples collected during application.

Using the estimated mean regression response as a point of reference, spore recovery profiles remained relatively uniform through the first 3 downstream sample stations with respect to the fol-

Figure 1 DISTRIBUTION of Bti DURING & AFTER TREATMENT APPLICATION at the 37m DOWNSTREAM SAMPLE STATION

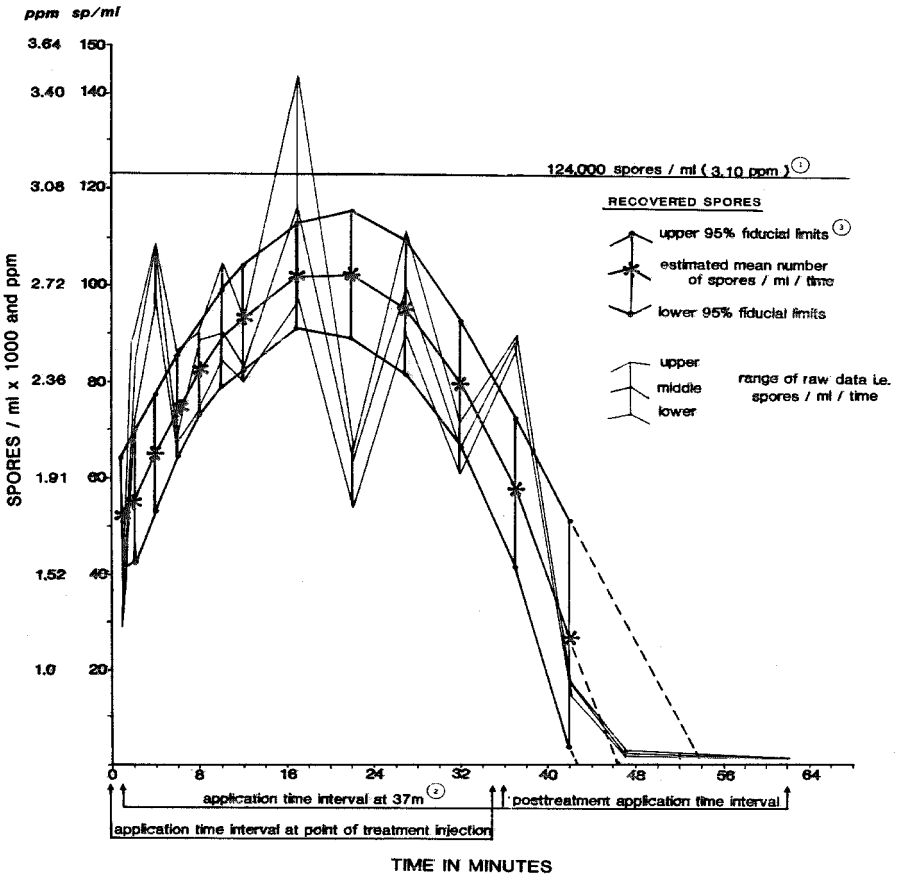

Fig. 1. 35 Min Exposure to 3.10 ppm *Bti*—24 HR Posttreatment

[1] Desired concentration level of *Bti* to be maintained in test stream for 35 minutes.
[2] One and one-tenth minutes for leading edge of *Bti* treatment concentration to arrive at 37 m, i.e., 0.55 ± 0.12 m/sec mean stream flow rate.
[3] Estimated mean spores/mL determined by polynomial regression analysis ($\tilde{\gamma} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 T^3$) where T = Time.

Figure 2 DISTRIBUTION of Bti DURING & AFTER TREATMENT APPLICATION at the 91m DOWNSTREAM SAMPLE STATION

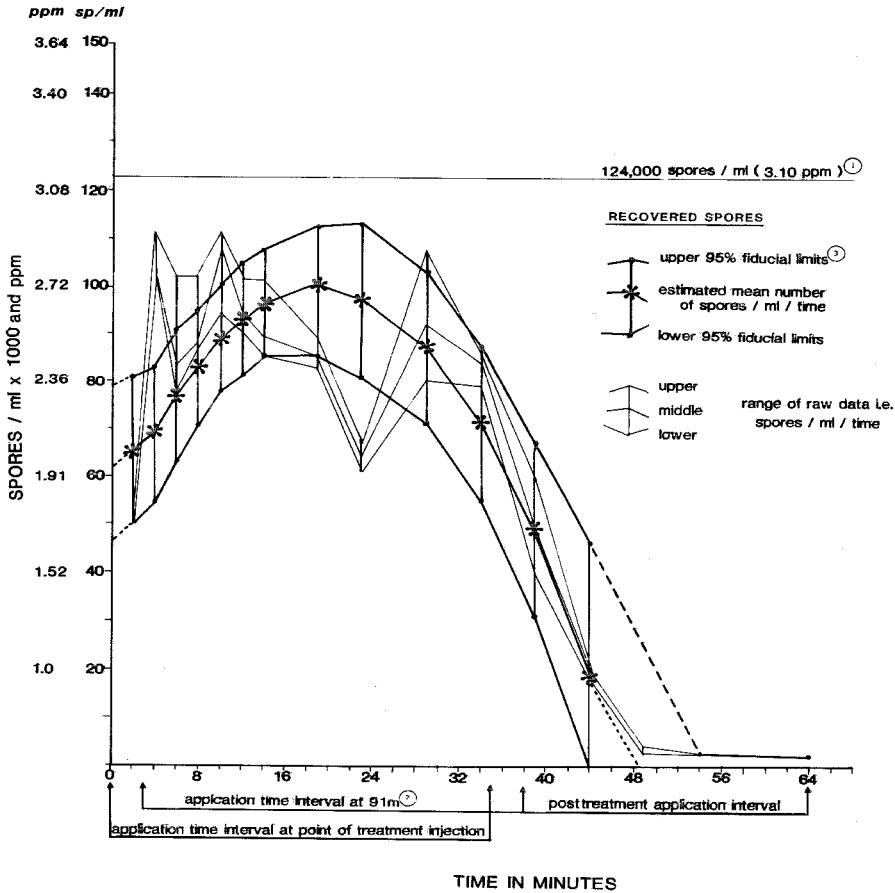

Fig. 2. 70 Min Exposure to 1.55 ppm *Bti*—24 HR Posttreatment

[1] Desired concentration level of *Bti* to be maintained in test stream for 35 minutes.
[2] Two and eight-tenths minutes for leading edge of *Bti* treatment concentration to arrive at 91 m, i.e., 0.55 ± 0.12 m/sec mean stream flow rate.
[3] Estimated mean spores/mL determined by polynomial regression analysis ($\tilde{y} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 T^3$) where T = Time.

Figure 3 DISTRIBUTION of Bti DURING & AFTER TREATMENT APPLICATION at the 152m DOWNSTREAM SAMPLE STATION

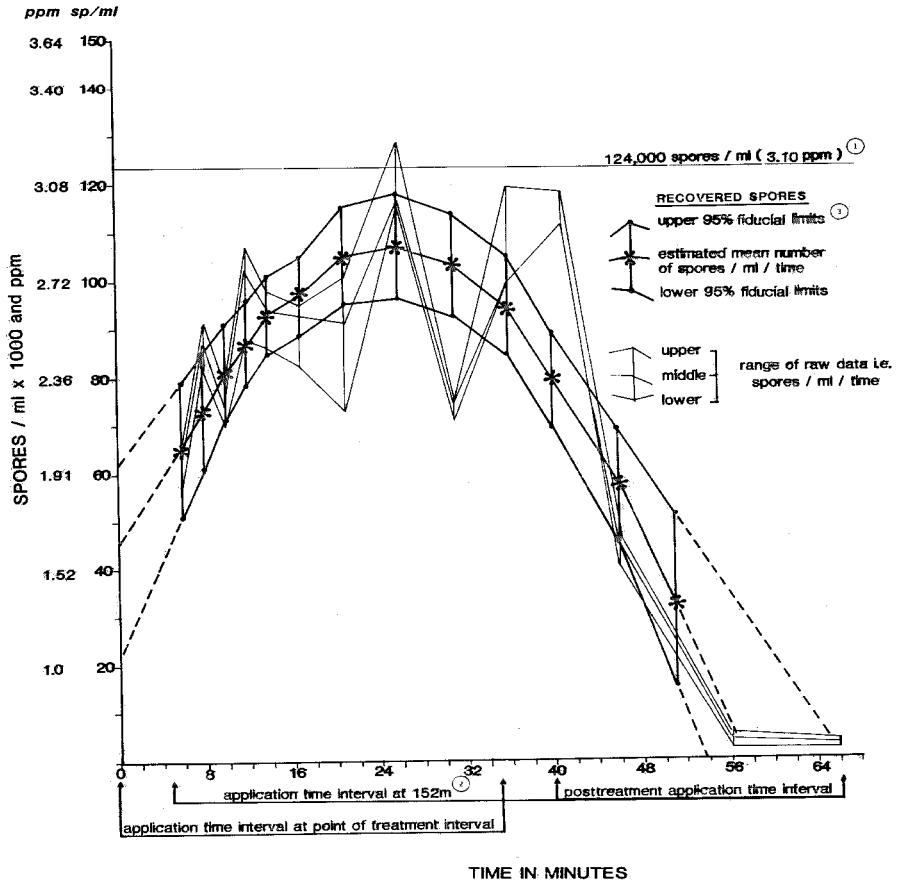

Fig. 3. 70 Min Exposure to 1.55 ppm *Bit*—48 HR Posttreatment

[1] Desired concentration level of *Bti* to be maintained in test stream for 35 minutes.
[2] Four and six-tenths minutes for leading edge of *Bti* treatment concentration to arrive at 152 m, i.e., 0.55 ± 0.12 m/sec mean stream flow rate.
[3] Estimated mean spores/mL determined by polynomial regression analysis ($\hat{y} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 T^3$) where T = Time.

Figure 4 DISTRIBUTION of Bti DURING & AFTER TREATMENT APPLICATION at the 312m DOWNSTREAM SAMPLE STATION

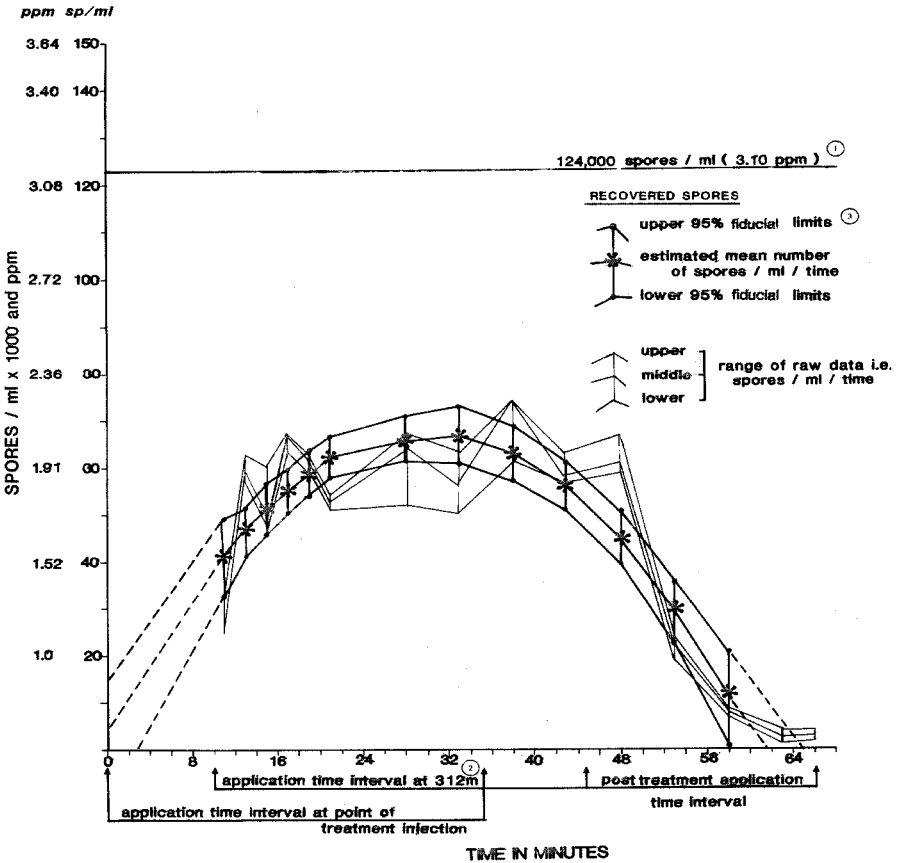

Fig. 4. 70 Min Exposure to 1.55 ppm *Bti*—72 HR Posttreatment

[1] Desired concentration level of *Bti* to be maintained in test stream for 35 minutes.
[2] Nine and one-half minutes for leading edge of *Bti* treatment concentration to arrive at 312 m, i.e., 0.55 ± 0.12 m/sec mean stream flow rate.
[3] Estimated mean spores/mL determined by polynomial regression analysis ($\tilde{y} = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 T^3$) where T = Time.

lowing: (a) the difference between initial treatment concentration level prior to application from that occurring during application, (b) time to peak concentration, (c) spread of upper and lower fiducial limits per time-distance, (d) concentration spread per application time interval, i.e., 35 min, and (e) rate of ascent and descent of spore concentrations following the initiation and termination of treatment application, respectively.

The most significant difference from the first 3 sample stations from that of the last (312 m) was the appreciable reduction in overall recovered spores.

Using the estimated mean regression line in Figures 1 to 4, 50 to 80% (1.5 to 2.5 ppm) of the desired 3.10 ppm treatment concentration was recovered over a 20 to 22 min time interval, with peak recovery occurring approximately midway through the 35 min exposure time. The remaining 13 to 15 min consisted of the leading and trailing edges of the treatment suspension which were at noticeably reduced spore levels.

The difference between initial treatment concentration levels from that recovered may be a result of statistical variability in sample collecting procedures, i.e., single point samples instead of multiple samples per time-distance, and in laboratory spore determination procedures. Also, losses of *Bti* spores through settling or attachment, or dilution of the spore suspension by mixing with greater quantities of water, could account for the sudden drop in peak concentration at 312 m following stable profiles to 152 m.

Even if desired treatment levels are unattainable they still may be sufficient enough to produce high larval mortality, as noted from results of the field efficacy test conducted in May (Frommer et al. 1981) where 25 to 80% mortality was achieved respectively over a 312 m length of test stream. Little, if any, spore residual remained following treatment, as evident, at all sample stations, by the rapid decline in recovered spores once application has been terminated. Additionally, samples collected from several stream eddies 24 hr and 48 hr following treatment produced residual spore counts of less than 100/ml, which is far below the level of lethal activity.

The variability in recovered spore concentrations may result from several sources: (a) statistical variability in sample collecting procedures, (b) statistical variability in preparing and determining spore counts, (c) variability in formulating *Bti* for field use, i.e., suspension of spores in the stock solution prior to dispensing, and (d) nonrandom movement of spores within the stream.

Dutka and Kwan (1980) best explained the phenomenon of spore distribution; they indicated that bacteria do not form a complete homogeneous suspension with water, but tend to act as particulates precipitating to the sediment or are adsorbed to larger floating particles or caught in eddies, then later released or resuspended from the sediment to be carried farther downstream.

An important factor to consider during treatment is if major physical obstacles are part of a stream, then they must be accounted for. Though not tested in this study, it would be fair to assume if significant physical obstructions existed, then the possibility of suspension accumulation could lead to prolonged leaching of spores over extended periods of time, far beyond the initial desired exposure time. Thus, a 35 min exposure could potentially expand to several hours or longer at sublethal concentration levels, preventing any effective reduction in larvae. Likewise, accumulation and leaching could create the effect of a natural slow release mechanism whereby lethal levels, though below initial treatment concentrations, could effectively reduce larval populations.

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