THE EFFECTS OF EXTENSIVE AQUATIC VEGETATIVE GROWTH ON THE DISTRIBUTION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* IN FLOWING WATER[1]

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ABSTRACT. Test data from 2 field trials showed that extensive growth of *Potomogeton crispus* and *P. pectinatus* had varying effects on the movement of *Bacillus thuringiensis* var. *israelensis (Bti)* through a 312 m section of test stream. During one 35 min exposure time, 24 to 90% (0.8 to 2.8 ppm) of a 3.10 ppm *Bti* treatment concentration was recovered over an 18 to 28 min period. However, during a second field trial, suspected stream channelization resulted in a significant increase in recovered spores ranging from 62,000 to 205,000 spores/ml (1.9 to 6.4 ppm, i.e., 61 to 206%).

INTRODUCTION

In early May 1980, this laboratory initiated a study at Holston Army Ammunition Plant (HAAP), Kingsport, TN, to investigate the effects of flowing water, from a moderately sized stream containing no extensive aquatic vegetative growth, on the maintenance of *Bacillus thuringiensis* var. *israelensis* de Barjac (*Bti*) at a desired concentration level for a specific period of time over a known distance. The distribution of *Bti* showed that during a 35 min application 50 to 80% (1.5 to 2.5 ppm) of the desired 3.10 ppm *Bti* treatment concentration was recovered over a 20 to 22 min interval, with peak recovery occurring midway through the initial exposure period (Frommer et al. 1981b).

With the dense growths (nearly 90% coverage) of the aquatic weeds *Potomogeton crispus* L. and *P. pectinatus* L. occurring from midsummer to early fall, the parameters influencing the distribution and dissipation of *Bti* suspensions changed. These weeds not only provided additional attachment substrates, but also could act to alter the movement of spores through filtering, channeling, and/or delaying treatment suspensions.

The current study was conducted in July and September 1980 at HAAP to investigate what effects dense growths of *P. crispus* and *P. pectinatus* have on the distribution and dissipation of *Bti*.

METHODS AND MATERIALS

The *Bti* used was an experimentally formulated powder provided by Abbott Laboratories (Lot No. 6478-194) with an International Toxic Unit (ITU)/mg rating of 800–1200. A 402 m section of the test stream with no major physical obstacles, i.e., sharp bends, deep stream bed depressions, or constrictions in stream width, was selected as the test area. In the first field trial conducted in July, the test stream ranged from 3.0 to 3.6 m in width and from 20 to 50 cm in depth, with a flow rate and volume at 0.46 ± 0.14 m/sec and 23,900 liters/min, respectively. However, during September the volume of water was significantly higher. The test stream in this instance ranged from 3.0 to 3.6 m in width and from 45 to 60 cm in depth, with the flow rate and volume at 0.49 ± 0.16 m/sec and 43,890 liters/min,

[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.

respectively. All flow rates were determined with a Gurley Pygmy Type Current Meter, Model 625. Flow in both tests was a combination of water from the plant and a natural stream that drained into the test area. Procedures used for treatment application and for monitoring spore distribution profiles were identical in both field trials. Treatment consisted of producing a 3.10 ppm stream concentrate of *Bti*, i.e., an estimated 98,000 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. Considerable caution should be exercised when making comparisons, since these ratings are established from results of bioassays using *Aedes aegypti* (Linn.) larvae and not with simuliid larvae. Unpublished bioassay tests conducted by this laboratory using *Simulium vittatum* Zetterstedt showed there was little difference in the larvicidal activity between these 2 lots of *Bti*. Weight/volume expressions (ppm) were correlated to corresponding spores/ml using procedures described by Frommer et al. (1980). 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 is 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 for monitoring the distribution of *Bti* was identical to the work previously conducted by Frommer et al. (1981a).

RESULTS AND DISCUSSION

Figures 1 to 4 illustrate the distribution and dissipation pattern of spores collected during the July field trial. All pretreatment background spore counts of this trial 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 uni-

[1] Desired concentration level of *Bti* to be maintained in test stream for 35 min.

[2] 0.9 min for leading edge of *Bti* treatment concentration to arrive at 37 m, i.e., 0.46 ± 0.12 m/sec mean stream flow rate.

[3] 2.7 min for leading edge of *Bti* treatment concentration to arrive at 91 m, i.e., 0.46 ± 0.12 m/sec mean stream flow rate.

[4] 5.2 min for leading edge of *Bti* treatment concentration to arrive at 152 m, i.e., 0.46 ± 0.12 m/sec mean stream flow rate.

[5] 12.7 min for leading edge of *Bti* treatment concentration to arrive at 312 m, i.e., 0.46 ± 0.12 m/sec mean stream flow rate.

[6] 1.2 min for leading edge of *Bti* treatment concentration to arrive at 37 m, i.e., 0.50 ± 0.14 m/sec mean stream flow rate.

[7] 2.9 min for leading edge of *Bti* treatment concentration to arrive at 91 m, i.e., 0.50 ± 0.14 m/sec mean stream flow rate.

[8] 5.2 min for leading edge of *Bti* treatment concentration to arrive at 152 m, i.e., 0.50 ± 0.14 m/sec mean stream flow rate.

[9] 10.6 min for leading edge of *Bti* treatment concentration to arrive at 312 m, i.e., 0.50 ± 0.14 m/sec mean stream flow rate.

[10] 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).

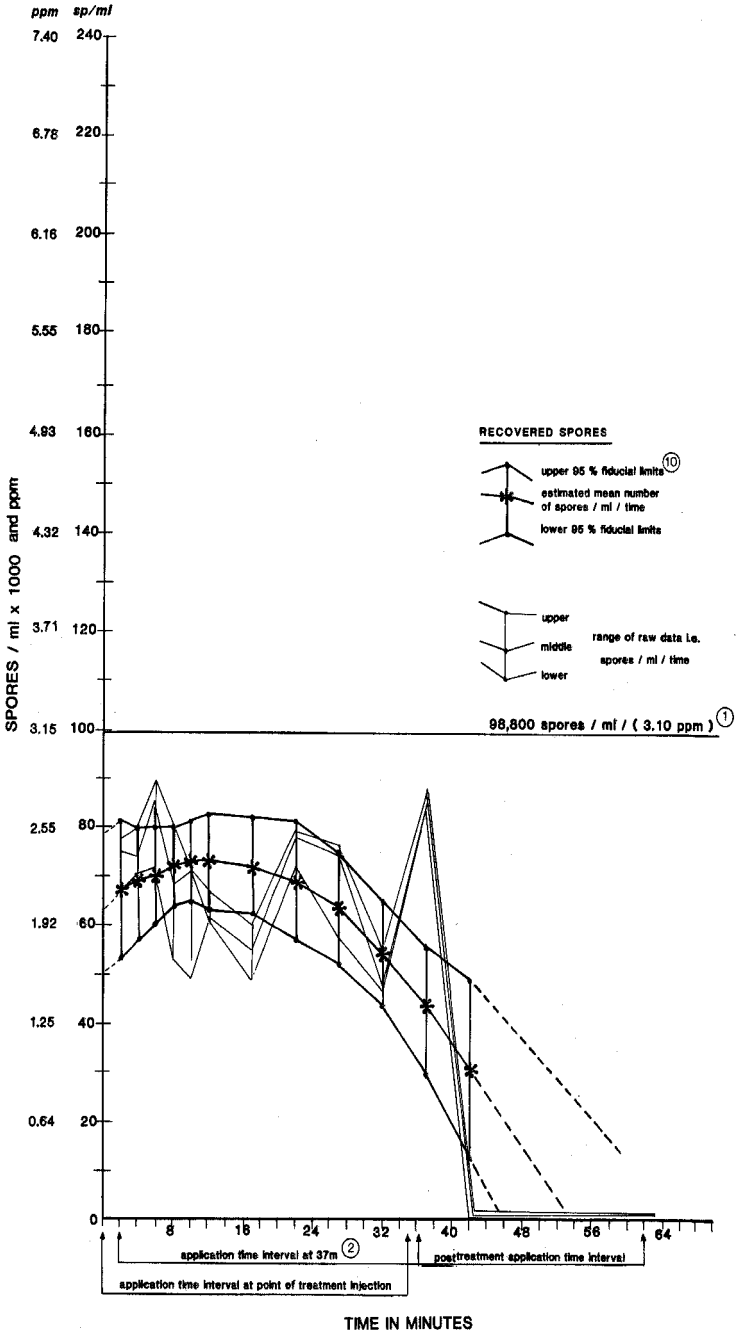

Fig. 1. Distribution of *Bti* during and after treatment application at the 37 m downstream sample station (July field trial).

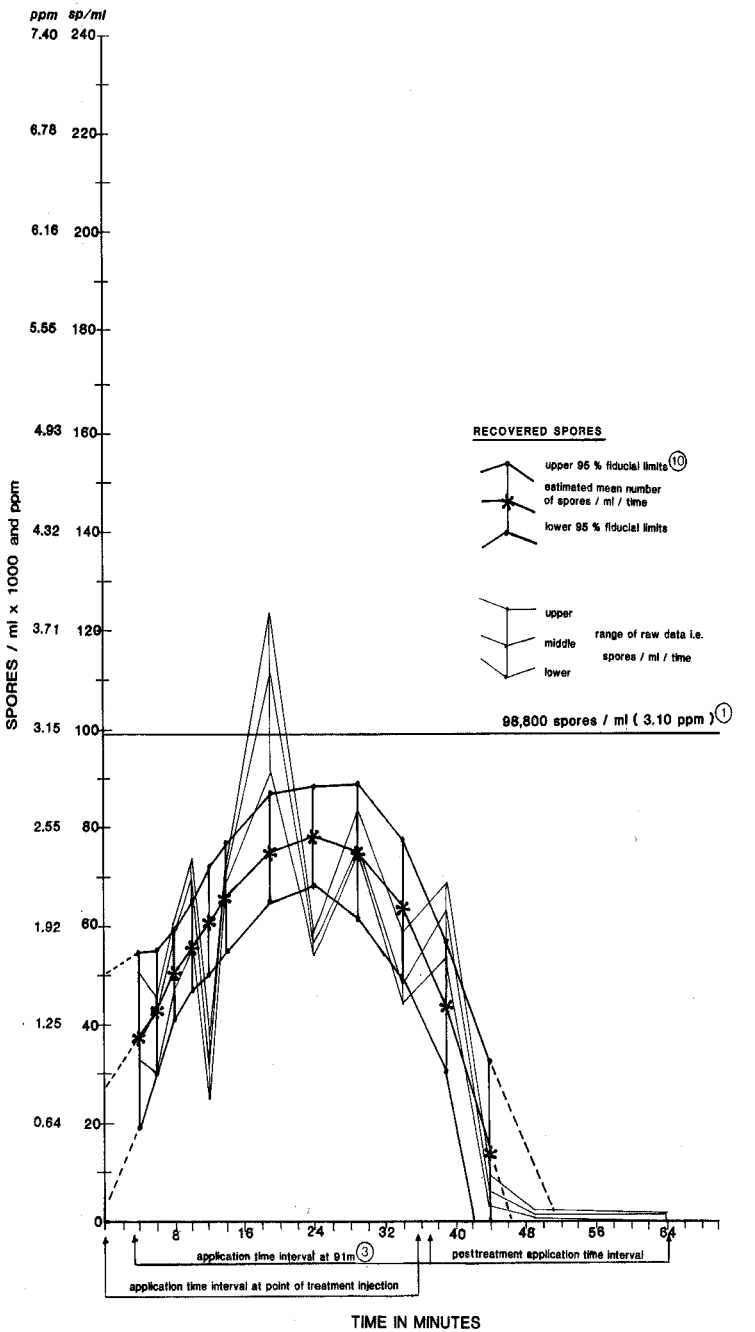

Fig. 2. Distribution of *Bti* during and after treatment application at the 91 m downstream sample station (July field trial).

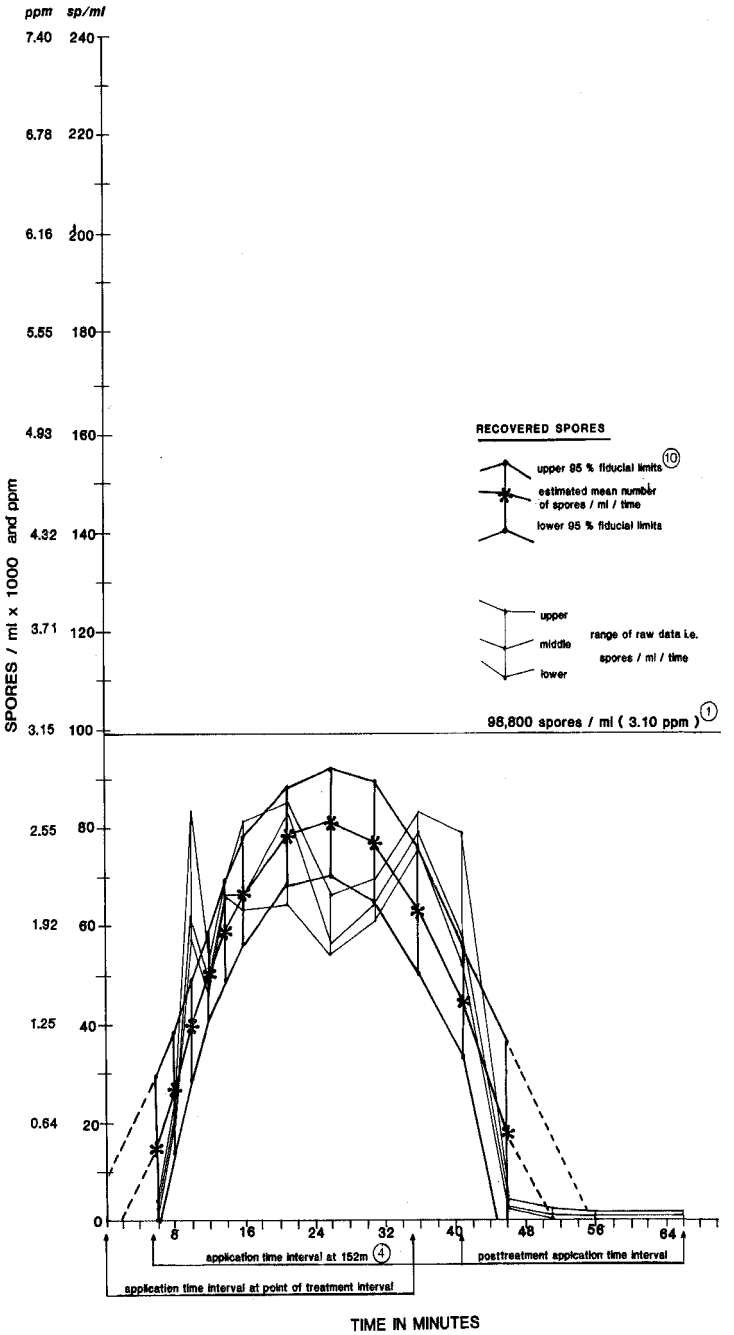

Fig. 3. Distribution of *Bti* during and after treatment application at the 152 m downstream sample station (July field trial).

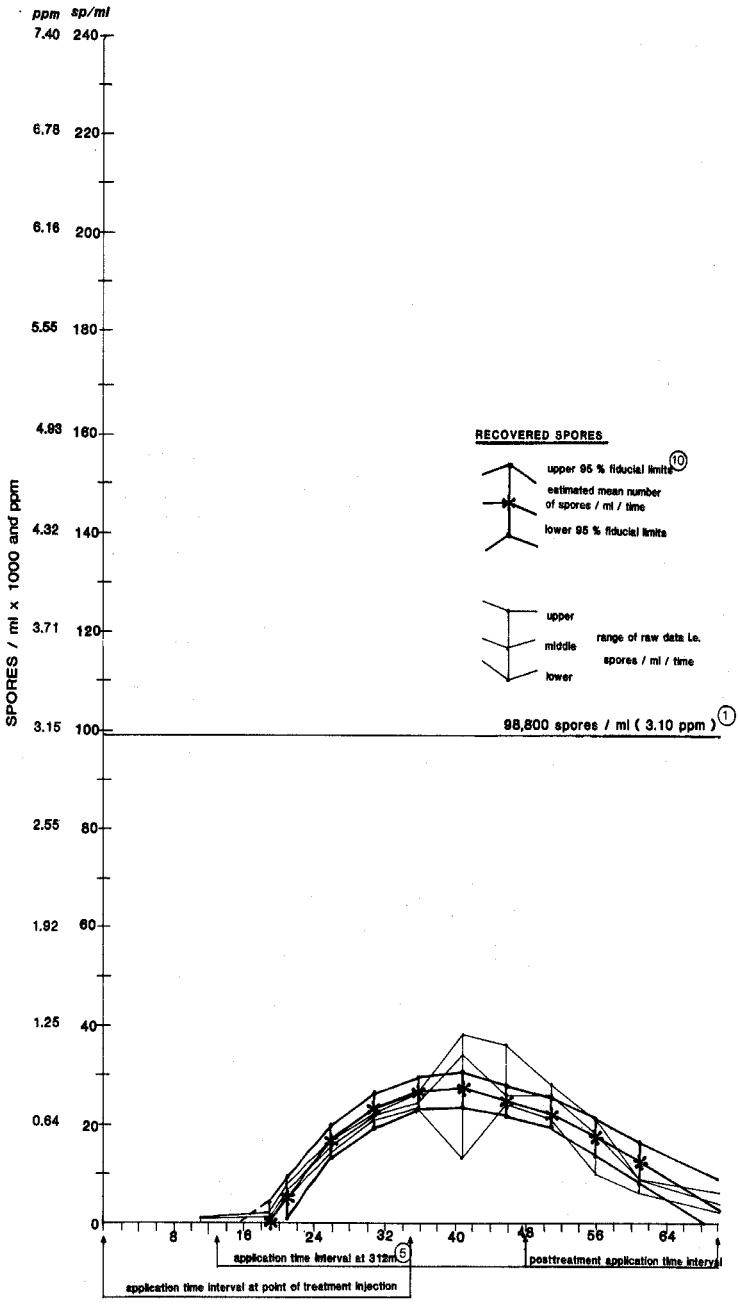

Fig. 4. Distribution of *Bti* during and after treatment application at the 312 m downstream sample station (July field trial).

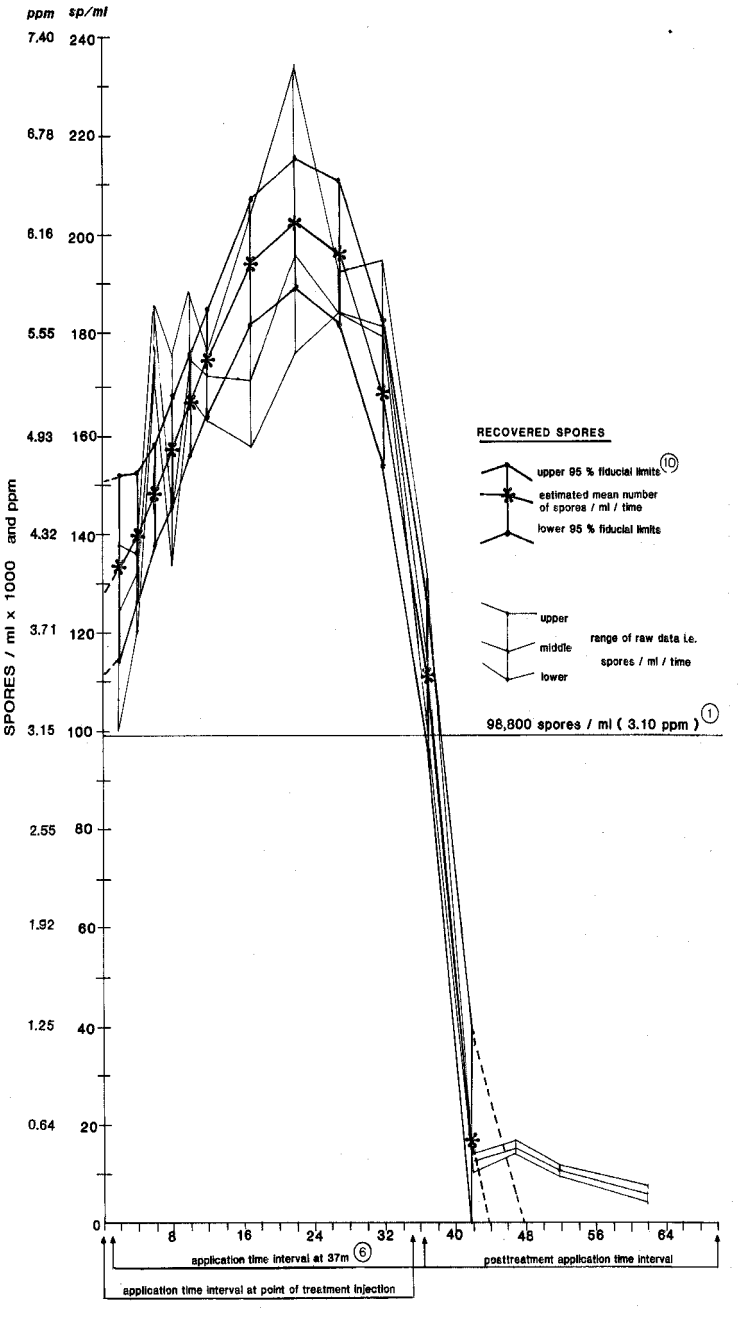

Fig. 5. Distribution of *Bti* during and after treatment application at the 37 m downstream sample station (September field trial).

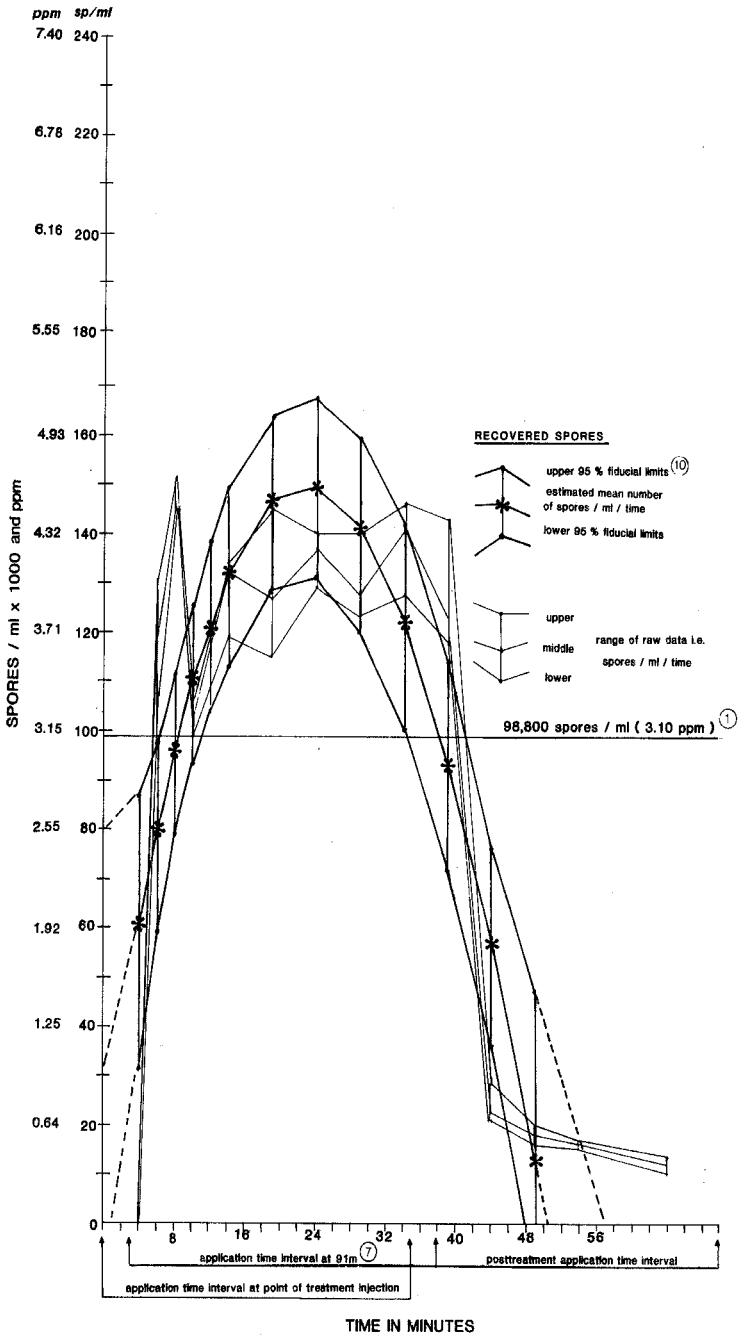

Fig. 6. Distribution of *Bti* during and after treatment application at the 91 m downstream sample station (September field trial).

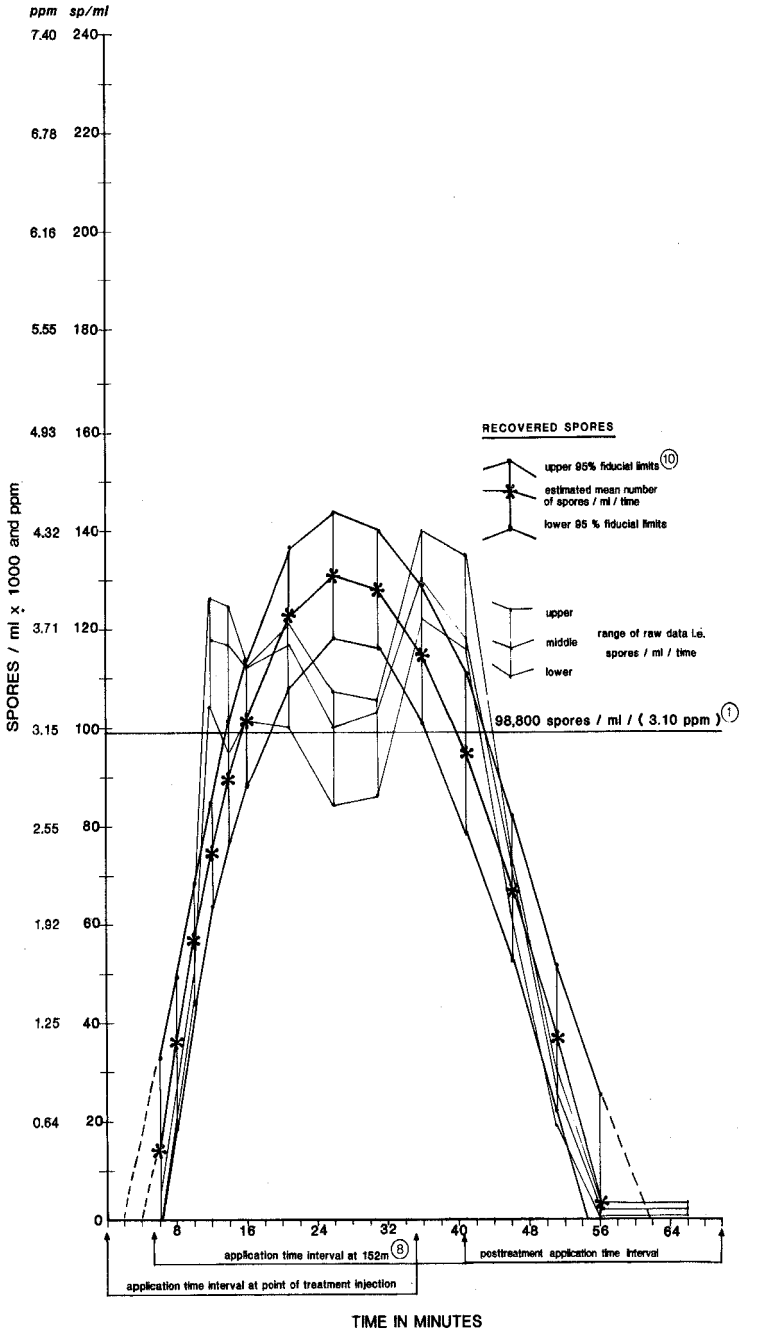

Fig. 7. Distribution of *Bti* during and after treatment application at the 152 m downstream sample station (September field trial).

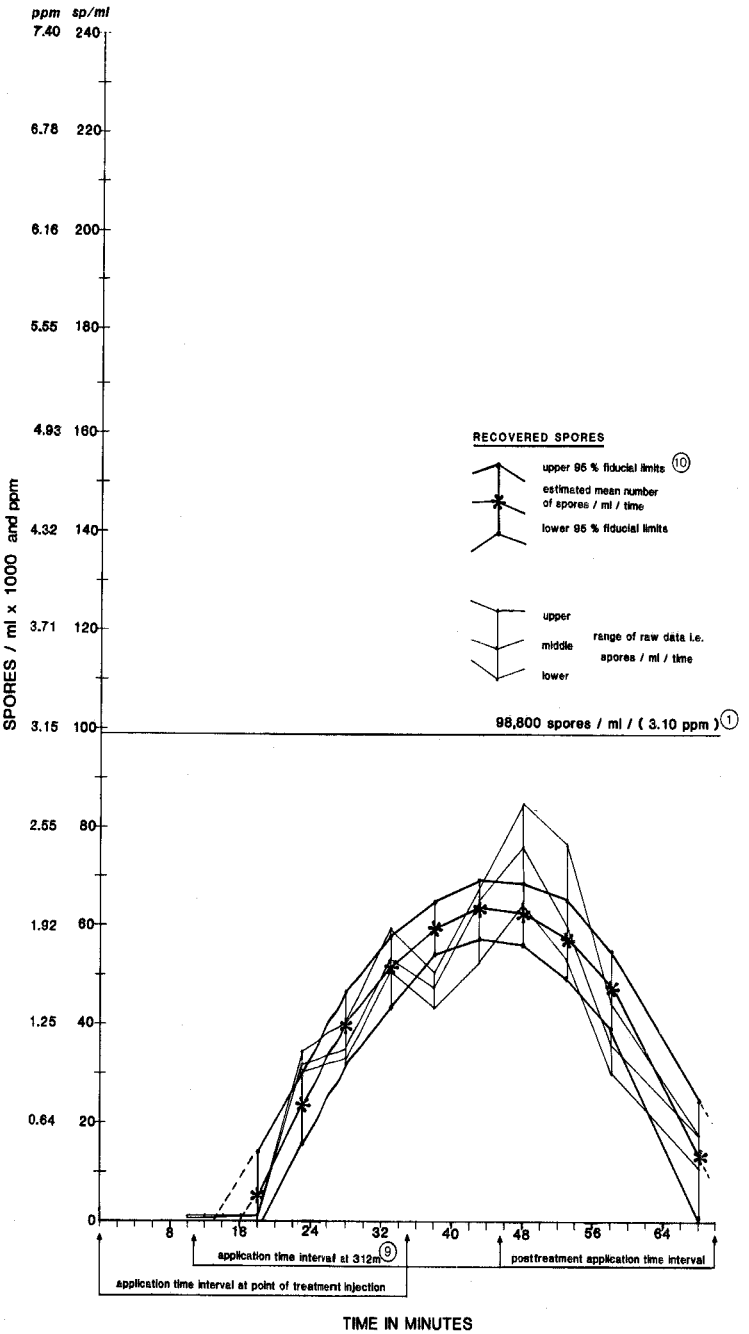

Fig. 8. Distribution of *Bti* during and after treatment application at the 312 m downstream sample station (September field trial).

form through the first 3 downstream sample stations (Figs. 1–3) with respect to the following: (a) differences between initial treatment concentration level (98,000 spores/ml, i.e., 3.10 ppm) prior to application from that occurring (recovered) during application (88,000 to 55,000 spores/ml, i.e., 2.8 to 1.8 ppm); (b) time to peak concentration (18 to 22 min); (c) spread of upper and lower fiducial limits (± 10,000 spores/ml) per time-distance; (d) delay in the arrival of treatment suspension at downstream sample stations (1 to 6 min); and (e) changes in the rate of ascent and descent of spore levels. The exception to this uniformity was at 37 m where the rate of ascent had nearly peaked by the time the first spore sample was collected.

The most significant difference between the first 3 sample stations (Figs. 1–3) and the last (312 m; Fig. 4) was the last had a maximum spore recovery of only 24% (23,700 spores/ml) which occurred midway (22 min) through the 35 min treatment interval. Other differences at 312 m were smaller confidence bands (± 4,000 spores/ml) on fiducial limits, reduced rates of ascent and descent of spore levels, and an 11 min delay in the arrival of treatment suspension.

The spore profile presented here is quite similar in its overall pattern to the distribution study conducted in May 1980 (Frommer et al. 1981b) where extensive aquatic vegetative growth was absent.

The results from these test data showed that extensive aquatic vegetative growth did not significantly impair spore movement, i.e., spore profile integrity maintained close to a 35 min application interval, or larvicidal activity, as demonstrated by the field efficacy test conducted in July 1980 where a 27 to 92% reduction in larvae was achieved (Frommer et al. 1981c).

The distribution and dissipation of spore patterns collected from the September field study are illustrated in Figs. 5–8. As in the July field study, all pretreatment background spore counts were at insignificant levels.

Following the estimated mean regression line as a point of reference, spore recovery profiles varied drastically between each sample station with reference to (a) the difference between initial treatment concentration level prior to application; (b) time to peak concentration; (c) spread of the confidence bands (± 10,000 spores/ml) on the 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.

At 37 m (Fig. 5), the peak level of recovered spores occurred 18 to 24 min into the 35 min application interval. The amount recovered was 205,000 spores/ml, slightly more than twice the amount (98,800 spores/ml) initially applied. No noticeable delay in the arrival of the treatment suspension was observed. However, there was a slight change in the rate of descent as reflected in the trailing edge of the spore suspension profile where a 2 to 4 min increase in the 35 min application period was recorded.

As the treatment suspension moved to 91 m (Fig. 6), the peak level of spore recovery dropped to 145,000 spores/ml and remained at this level through 152 m (Fig. 7). Again, peak spore recovery at both sample stations occurred midway through the 35 min treatment interval.

Only at 312 m (Fig. 8) does the peak level of recovered spores (62,000/ml) fall below the level initially dispensed. As noted, not only were the rates of ascent and descent of the estimated suspension profile measurably suppressed from those observed at the 3 previous sample stations, but there was an 11 to 12 min delay in their arrival.

A possible explanation for the dissimilar profile in recovered spore counts in the September field trial as compared to the July trial is the increased volume of water in conjunction with extensive aquatic vegetative growth. This combined physical effect apparently channeled, through the first 2 sample stations (37 and 91 m), much of the spore suspension

into a smaller volume of water, thus producing a higher recovered spore count from that initially calculated prior to treatment. The gradual drop in recovered spores at 152 m was most likely caused by greater spore dispersion as the channeling effect lessened.

In both the July and September field trials, losses of *Bti* spores through settling or attachment or dilution of the spore suspension by mixing with greater quantities of water may have accounted for the sudden drop in peak concentration at 312 m.

Even if desired treatment levels are unattainable, they still may be sufficient enough, assuming proper dosage, to produce high larval mortality as noted from results of field efficacy tests conducted in May and July 1980 where 25 to 80% and 27 to 92% mortality was achieved, respectively.

Results from both field trials indicate little, if any, spore residual remains 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 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, as discussed by Frommer et al. (1981b), may have resulted from several sources ranging from the statistical variability in sample collecting procedures to nonrandom movement of spores within the stream. However, in this instance, channelization caused by excessive vegetation in alliance with specific flow volumes of water may also have accounted for some of this variability.

Finally, caution should be taken when analyzing spore distribution patterns, since too few samples collected could result in an underestimation of actual downstream treatment spore levels.

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