# THE EFFICACY OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST BLACKFLY LARVAE (DIPTERA: SIMULIIDAE) IN THEIR NATURAL HABITAT

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ABSTRACT. Suspensions of *Bacillus thuringiensis* var. *israelensis* spores and crystals were tested as a simuliid larvicide in small Newfoundland streams. One minute dosages with initial concentrations of 1 x $10^5$ viable cells/ml caused up to 100% mortality in larval simuliids. The flow rate of the stream appeared to be a major variable in determining the maximum distance downstream from the dose site at which the treatment was still effective.

*Bacillus thuringiensis* var. *israelensis* de Barjac (*B.t.i.*) was found to be highly toxic to blackfly larvae in laboratory tests (Undeen and Nagel 1978, Undeen and Berl 1979). The running water habitat of blackfly larvae places a limit upon the dosage time in practical applications. Dosages of about 1 x $10^5$ vc/ml for 1 min, derived from earlier lab work (Undeen and Nagel 1978), were applied to small Newfoundland streams as a test for field efficacy.

## MATERIALS AND METHODS

*B.t.i.* was cultured on tryptose blood agar base (Difco) for 5–7 days at 30°C, scraped from the agar with a spatula and suspended in water. The numbers of viable cells/ml in the suspensions were estimated by a plate counting technique and the suspensions were stored at 5°C until used.

Stream flow was measured with an Ott propeller type flow meter with a 2 cm propeller to obtain the stream volume. Calculated dosages of 1 x $10^5$ viable cells (vc)/ml for 1 min of flow were administered from a sprinkling can which was calibrated to empty in 1 min. The dosage was applied at the head of a single channel riffle which was followed by only minor slow flowing reaches between riffles over the next 100–200 m of stream channel. During and after dosage, water samples were taken about 10 m below the dose site at 15 sec interval and brought back to the laboratory for assessment of the "applied dosage" by plate counts.

One to two hr after dosage, samples of larvae were collected at measured intervals downstream from the site of application while undosed control larvae were collected upstream from the application point. The larvae were brought back to the laboratory on ice, and live larvae from these samples were placed, 30 per container, on the rearing system (Colbo and Thompson 1978). For controls in tests 3 and 4, ten pre-dosage larva samples were collected from the same stream area and their 48 hr survival assessed. Water was changed and 25 mg of Tetra® fish food was added daily to each container and after 48 hr the surviving larvae were counted. Percentage mortality of the dosed larvae was corrected for undosed control mortality using Abbott's formula.

Field mortality was assessed by selecting stones, usually with the longest dimension between 10 and 20 cm, from areas normally colonized by simuliid larvae. All the larvae from 15–20 stones were removed by scrubbing the stones with a brush and preserved in alcohol for identification and counting. The area of each stone was roughly calculated and each count was corrected to a "standard stone" of 1000 $cm^2$. Samples were taken one day before dosing and 3–6 days post-dosage. The larval counts were compared using the Mann Whitney U test. The totals of all simuliid species are reported here as they are all of approximately equal susceptibility (Undeen and Nagel 1978).

## RESULTS

The blackfly species present in test #1 (July 78) were in the *Simulium verecundum* complex. During test number 2 there were late instars of *Prosimulium mixtum, Cnephia ornithophilia, Stegopterna mutata* and *Simulium vittatum* along with early instars of *Simulium venustum*. Only the *S. venustum/verecundum* complex, the *S. tuberosum* complex and *S. vittatum* were present in tests number 3 and 4.

The actual dosages of spores as measured by plate counts of the water samples, were fairly close to the 1 x $10^5$ calculated dosages (Table 1).

Table 1. Larval simuliid mortality resulting from a 1 minute dosage of *Bacillus thuringiensis* var. *israelensis* to a Newfoundland stream.

| Test | dosage in viable cells / ml stream water | Simuliid larvae/stone Pre-dosage | days between samples | Post-dosage | M.W. −U test |
|---|---|---|---|---|---|
| 2 | $7.7 \times 10^4$ | 241.9 | 7 | 207.9/7 | NS |
| 3 | $1.5 \times 10^5$ | 151.3 | 6 | 13.3/6* | S |
| 4 | $2.2 \times 10^5$ | 4 | 4 | 1.1/4** | S |

* Surviving larvae grouped near the dose site.
** Surviving larvae grouped at about 100 m below the dose site.

The 2 mortality assessment methods yielded very similar results, both indicating percentage mortalities of up to 100% in some dosed stream areas. The post-dosage surviving larvae in tests 3 and 4 (Table 1) were found mostly in samples taken from the stream areas in which laboratory mortalities were less than 100% (Figure 1). Laboratory mortalities in 10 samples collected the day before dosage were quite low, a mean of 6% (range 0–15%) before test #3 and also 6% (range 0–23%) before test #4.

## DISCUSSION

Since the dosage time relationship changes with passage downstream, there will be some stream areas in which an optimum would occur. Upstream, near the application site the bacterial count will be high but the time short (test 2) and the larval kill poor, while farther downstream the dosage will be spread out providing for a longer dosage time with a still-adquate dose. Eventually by removal and dilution the bacterial concentration is below the lethal level, even for prolonged exposures.

The results indicated that when the stream volume is high the downstream carry is greater than when the volume is low (Fig. 1). This is probably related to the surface/volume ratio as all of the attached organisms which filter finely particulate matter from the water have less total volume of water to filter and clear the water in a shorter distance. Large numbers of net feeding caddisflies in the families Philopotomidae and Hydropsychidae were present and algae were also much more abundant during tests 3 and 4 than earlier. This combination of fauna and flora probably was a significant filter at low stream flow, causing, if not actual reduction in bacterial concentration, a more rapid spread and dilution.

For mortality to occur it is essential that bacterial preparation be ingested by the larvae, and there is limited time during short exposures for them to ingest enough toxic material. If feeding is inhibited by some component of the formulation, the dose might pass by before feeding is resumed. It seems, therefore, that a black fly formulation of an ingestible toxicant must not contain any chemical which would be likely to irritate larvae.

From our laboratory observations on the effects of *B.t.i.* on simuliids attached

to stir-bars, there was no indication of the bacteria causing immediate larval detachment and drift, even though some larvae were moriband or dead within an hour after exposure. Of the field dosed larvae brought back to the laboratory for mortality measurement, only the live larvae were put into rearing containers, thereby introducing a bias towards the survivors. The stress of transfer from stream to laboratory could introduce an opposite bias possibly explaining the higher % mortality in the laboratory than in the field assessment in test #1. The larvae were removed from 3°C stream water and placed in 17° water in the laboratory, and, although corrected for control mortality, the combined effect of the bacteria and temperature shock could have increased mortality. However, downstream drift and the concentration of larvae due to the reduced stream level between the two sample collections (7 days) could also have accounted for the high post-dosage field larvae count. This is strongly indicated by a 22% increase in other stream insects (Colbo and Undeen 1980) while there was a 14% reduction of the simuliid larvae (Table 1).

It is clear from the above that more tests are required to quantify the effects of the various factors such as flow rate, temperature, biological activity and dosage on larval mortality. Nonetheless, the dosages used in these tests required only about 10 ml of "formulation," consisting of 1 x $10^{10}$ vc/ml, per cubic meter of stream water. This quantity compares favorably with the amounts of methoxychlor formulations required for treatment in the Athabasca River black fly abatement program (Shemanchuk pers. comm.) and of the temephos formulation used in the West African Onchocerciasis control program (Walsh et al. 1979). This, combined with its lower toxicity to non-

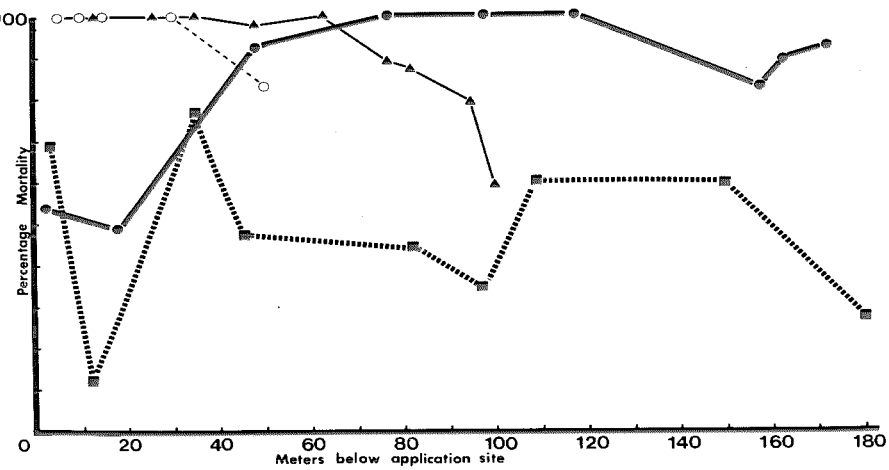


Figure 1. Corrected percentage mortality of simuliid larvae downstream from the dosage site. Larvae were dosed on their natural substrate and brought back to the laboratory for assessment of % mortality.

1. (○) 25 July 78, 19°C, 6.6 x $10^4$ vc/ml, 600 l/min. (Bauline Brook)
2. (■) 17 April 79, 3°C, 7.7 x $10^4$ vc/ml, 3,400 l/min. (Robins Pond Outlet)
3. (●) 6 June 79, 15°C, 1.5 x $10^5$ vc/ml, 2,000 l/min. (Robins Pond Outlet)
4. (▲) 8 August 79, 22°C, 2.2 x $10^5$ vc/ml, 200 l/min. (Robins Pond Outlet)

target organisms (Colbo and Undeen 1980) strongly recommends *B.t.i.* as a possible replacement for or supplement to these chemicals for control of black flies in their larval stages.

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