A TEST OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* DE BARJAC AS A LARVICIDE FOR *SIMULIUM OCHRACEUM*, THE CENTRAL AMERICAN VECTOR OF ONCHOCERCIASIS

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ABSTRACT. The efficacy of *Bacillus thuringiensis* var. *israelensis* against the larvae of *Simulium ochraceum*, the primary vector of onchocerciasis in Guatemala, and other simuliids was tested in small Guatemalan streams. One minute treatment with initial concentrations of 2 x 10^5 spores/ml resulted in up to 100% mortality but downstream carry was poor.

INTRODUCTION

There are currently 3 black fly abatement projects in operation. The greatest effort is the World Health Organization's Onchocerciasis Control Programme. It involves the control of *Simulium damnosum* s.l. (vectors of *Onchocerca volvulus*) in the Volta River Basin, West Africa, through the aerial application of Abate® (= temephos) to the breeding sites in rapids of large rivers. *Simulium arcticum*, another large-river species, is a serious pest of cattle on the Canadian prairies. Methoxychlor is being used with success against this species in the Athabasca River, Alberta. The 3rd project is a joint enterprise of The Guatemalan National Malaria Control Service and the Japanese Mission on Onchocerciasis Control in Guatemala, where *Simulium ochraceum*, the Central American vector of *O. volvulus*, breeds in small mountain streams with discharges of generally less than 300 liters per min. This environment presents an entirely different control situation from the other 2 projects. The Japanese/Guatemalan teams have developed a suitable methodology, which depends upon larviciding of streams with quick-dissolving temephos briquettes.

Bacillus thuringiensis var. *israelensis* (*Bti*), a pathogen isolated from mosquitoes in Israel (Goldberg and Margalit 1977), was also found to be toxic to Newfoundland black fly larvae in the lab (Undeen and Nagel 1978a) and in natural streams (Undeen and Colbo 1980). Laboratory tests in Ivory Coast in 1978 established that *Bti* is also toxic to *S. damnosum* (Undeen and Berl 1979). Subsequent tests (Guillet and de Barjac 1979) of R153–78 (a commercial *Bti* powder) have confirmed these results under more natural conditions. Comparisons made from the above reports and unpublished data reveal that fresh aqueous suspensions of spores and crystals are considerably more effective against simuliids than the powdered materials, emphasizing the importance of formulation. The Newfoundland experiments demonstrated a direct correlation between downstream carry and stream discharge (Undeen and Colbo 1980). The present study examines the efficacy of *Bti* against *S. ochraceum* in the field.

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MATERIALS AND METHODS

Bacillus thuringiensis var. *israelensis* was cultured on tryptose blood agar base (Difco) at 30°C for 4 days, scraped from the agar surface, suspended in distilled water and stored at 5°C until use. This aqueous suspension plus an experimental formulation, SAN 402 WDC obtained from Sandoz, Inc., Homestead, Florida, was carried to Guatemala for testing against simuliid larvae.

Field tests were conducted in streams within the Finca Media Monte, the Finca Nimaya, and in the Guachipilin area. Stream discharge was measured by directing the entire stream flow into a large plastic bag for a timed interval.

Artificial substrates of white plastic film were anchored to the stream bottom in such a way that the larvae could be easily counted without disturbing them. After 48 hr colonization period the larvae on each substrate were counted and the stream treated with an aqueous suspension of *Bti*. Posttreatment counts, on the same substrates, were made 48 hr later at Medio Monte and 24 hr later at Nimaya and Guachipilin.

Another sampling technique used locally, compares the number of larvae collected from natural substrates during a 5 min period before and after treatment, providing a measure of treatment efficacy. This method was also used in some tests and as a cross check for the artificial substrates.

The streams at Finca Medio Monte originated from a spring, providing an *S. ochraceum* breeding site 14.5 m in length from the stream origin, terminating at a rock face upon which the water spread out and dropped 5 m. At the time of treatment the discharge was 10 liters/min and the temperature 19°C. A 1 min application of the aqueous spore suspension at the rate of 2 x 10^5 spores/ml was administered just below the origin. The predominant larval substrate was fallen leaves.

The Nimaya stream was treated at 5 sites with a dosage of 2 x 10^5 sp/ml at the top sites (A and B, Fig. 1) and 1 x 10^5 sp/ml at points (C, D, and E) where the discharge was higher. Twenty-four hr pretreatment, 15 stones were removed from the area between A and B, all the organisms brushed from these and preserved in alcohol. Simuliid larvae from these stones were identified and counted. The remaining nontarget organisms still await identification in connection with the related environmental impact assessment. Posttreatment stone samples were collected in the same way 24 hr later from the upper 60 m of the A-B zone. The artificial substrate method was also used between points A and B. Five min collections were made immediately before and 24 hr after treatment in the area numbered 1 through 5 on Fig. 1.

Area A-B had a lower discharge, larger number of pools, and more vegetation than the lower test segments.

The stream at Guachipilin originated at a spring less than 1 m above the treatment site. In the first 25 m, fallen leaves provided the most abundant larval attachment site, while the remainder of the test area ran through dense, tall grass with larvae attached to its roots and leaves. The stream temperature was 22°C

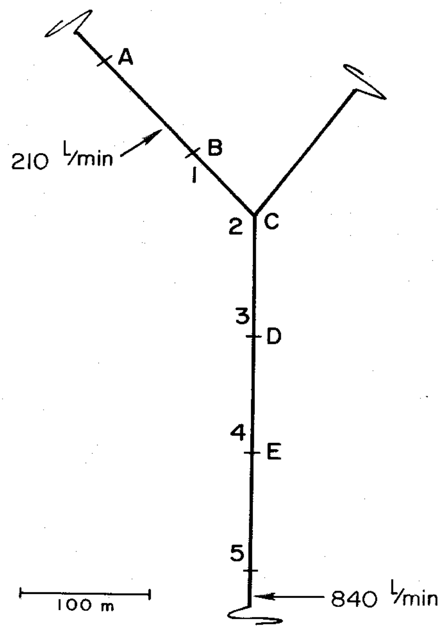

Fig. 1. Finca Nimaya treatment sites (A–E) and collection sites (1–5).

and its discharge 156 liters/min, measured 72 m below the treatment point. The Sandoz formulation was administered at a rate of 1 x 10^6 sp/ml for 10 min.

RESULTS

In the stream of Medio Monte, percentage mortality on 6 substrates ranged between 82 and 100% with a mean of 92%. No trend toward a decline in mortality was observed at the most distant sites, indicating a downstream carry of at least 14 m. A pretreatment 5 min collection from natural substrates yielded 67 *S. ochraceum*, 30 *S. metallicum*, and 13 *S. callidum* while no larvae were found during the posttreatment collection.

Consistently high larval mortality was observed on artificial substrates in the upper 50 m at the Nimaya stream segment A-B (Table 1, Fig. 1). Five substrates implanted min before treatment collected a total of 7 larvae, and the upstream check increased from 16 to 45 larvae during the 24 hr between treatment and the post-treatment count, implying that recolonization by drift from above the treatment site and not survival was responsible for some of the posttreatment count. In order of their abundance in the stone sample were *S. metallicum, S. callidum, S. ochraceum,* and *S. samboni*, with a total count of 165 pretreatment and 3 posttreatment.

Simulium callidum, S. metallicum, S. samboni, S. paynei, and an unnamed *Simulium* species were found in the 5 min collections below the treatment sites B, C, D, and E. Overall percentage mortalities were 97% at the upper and 87% at the lower ends of B and 83, 80, and 90% at the lower ends of C, D, and E, respectively (Fig. 1).

Larval mortality on artificial substrates was total in the upper 25 m of the Guachipilia stream and essentially nil throughout the rest of the sampled area (Table 1). By the 5 min collection method, mortality in the top 10 m was 100%, 60% between 15–30 m, and 62% at 50–60 m below the treatment site. Present, in order of their relative abundance, were *Simulium* sp., *S. metallicum,* and *S. ochraceum.*

DISCUSSION

Control of onchocerciasis in Guatemala involved treatment of small streams, like the one at Medio Monte, which are the primary breeding sites of *S. ochraceum*. The sampling techniques were dictated by these small streams, allowing for only a linear array of substrates in a larval breeding site.

The regions in which control was achieved were marked by substrates uniformly without, or with only a few, larvae

Table 1. Percentage mortality in simuliid larvae on artificial substrates from a single treatment with *B. thuringiensis* var. *israelensis*.

Meters from treatment site	Number of substrates	Pretreatment count	Posttreatment count	Percent mortality
Nimaya (one minute, 2 × 10^5 spores/ml)				
−.5m (above treatment)	1	16	45	0
0–25	4	233	8	97
25–50	3	71	13	82
50–75	5	171	83	51
75–100	5	80	63	21
Guachipilin (10 minutes, 1 × 10^6 spores/ml)				
−.1m (above treatent)	1	12	13	0
0–25	13	193	0	100
25–50	10	239	208	13
50–75	5	71	134	0

posttreatment. Farther downstream, where the treatment failed to carry, posttreatment/pretreatment count ratios varied widely between substrates. Surviving larvae could migrate between natural and artificial substrates between counts and, in streams as small as these, minute alterations of flow characteristics would affect its suitability of a substrate for larval attatchment.

The larvae found on the substrates implanted just prior to treatment and the increase in numbers on the substrate a few centimeters above the treatment point, indicate that recolonization was responsible for some of the apparent "survival."

Treatment at 100 m intervals appeared to provide control throughout the B-E area of the Nimaya streams. Some of the posttreatment larvae found were probably from upstream of A, the untreated tributary shown on Figure 1 and, perhaps, the lower region of A-B through which the treatment at A failed to carry. Higher discharge, fewer pools, less vegetation, and/or overlap of treatments might have been responsible for the superior carry in this area.

Simuliid larvae in the test streams were killed by *Bti* at dosages similar to those found effective in Newfoundland (Undeen and Colbo 1980), but downstream carry was shorter. Some factors expected to reduce carry are pools, which dilute the dosage and promote settling, vegetation and other obstructions, which increase the total surface area upon which the particles can be filtered from the stream, and reduced discharge, which increases the water-substrate interface ratio, again resulting in increased opportunity to filter the active material from the stream.

The failure of the high dosage used at Guachipilin to increase downstream carry was probably not due to the Sandoz formulation which, according to recent lab tests against *Simuliium verecundum* by one of us (A.U.), is as effective as our laboratory-produced bacteria, but to the filtration capacity of the stream. Although insufficient to prove the point, data from Newfoundland and Guatemala show downstream carry to be more positively correlated with discharge than with dosage.

In conclusion, *Bti* is toxic to *S. ochraceum* and other simuliids in the same habitat but because of low downstream carry, treatment intervals would have to be short to achieve control.

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References Cited

Goldberg, L. J. and J. Margalit. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergenti, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti*, and *Culex pipiens*. Mosquito News 37:355–358.

Guillet, P. and H. de Barjac. 1979. Toxicity of *Bacillus thuringiensis* var. *israelensis* for black fly larvae, vectors of onchocerciasis. Compte Rendus Hebdomadaires des Séances de l'Academie des Sciences Série D 289:549–552.

Undeen, A. H. and D. Berl. 1979. Laboratory studies on the effectiveness of *Bacillus thuringiensis* var. *israelensis* de Barjac against *Simulium damnosum* (Diptera: Simuliidae) larvae. Mosquito News 39:742–745.

Undeen, A. H. and M. C. Colbo. 1980. The efficacy of *Bacillus thuringiensis* var. *israelensis* against black fly larvae (Diptera: Simuliidae) in their natural habitat. Mosquto News 40:181–184.

Undeen, A. H. and W. L. Nagel. 1978. The effect of *Bacillus thuringiensis* ONR-60A strain (Goldberg) on *Simulium* larvae in the laboratory. Mosquito News 38:524–527.