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Field Evaluation of *Bacillus thuringiensis* var. *israelensis* for Control of Black Flies in the North Littoral Zone of Brazil's São Paulo State[1]

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The impact of three flowable concentrate formulations of **Bacillus thuringiensis** *var.* **israelensis** *(H-14) upon* **Simulium pertinax** *larvae was evaluated in 24 coastal streams of Brazil's São Paulo State. While no significant differences were found regarding the three formulations' effectiveness, significant correlations were found between the discharge rates of individual streams and the distances over which at least two of the formulations were carried effectively downstream to produce 80% mortality. The relatively short carry distances found for small streams could pose difficulties for control programs that need to treat large numbers of such streams, and suggests a need for research directed at increasing the distances* **B. thuringiensis** *(H-14) formulations can be carried.*

The North Littoral Zone of São Paulo State includes a stretch of land that runs from Serra do Mar (the state's coastal mountain range) to the Atlantic Ocean (Figure 1). Serra do Mar has a very irregular topography covered by very dense vegetation typical of this tropical region. There are a great number of rivers, streams, and creeks in the area, the majority being small or midsized. For the most part the water in these streams is clear and has a temperature around 22°C. The streams' courses are rough, punctuated by small falls and eddies, and they provide breeding sites of the sort preferred by *Simulium pertinax*.

This region has a resident human population of about 200,000 inhabitants, most of whom live in four small cities. The local economy depends almost entirely on tourism—as suggested by the fact that the summer (January–March) influx of tourists into these towns raises the local population by about 200%.

However, with the advance of urban development in the region, and the consequent clearing of vegetation in the area around the foot of the Serra do Mar Range, the area inhabited by black flies has grown considerably in recent years. This has had a serious negative impact on tourism, due to the discomfort caused by allergic reactions to black fly bites and the fact that these allergic reactions are most marked among the visiting tourists, who tend to be considerably more sensitive to bites than permanent residents.

In 1957 the Health Secretariat for the State of São Paulo began a black fly control operation using the organochlorine insecticides DDT and BHC. Later, in 1971, the program began applying the organophosphate temephos (Abate 500-E-Cyanamid). At present, control opera-

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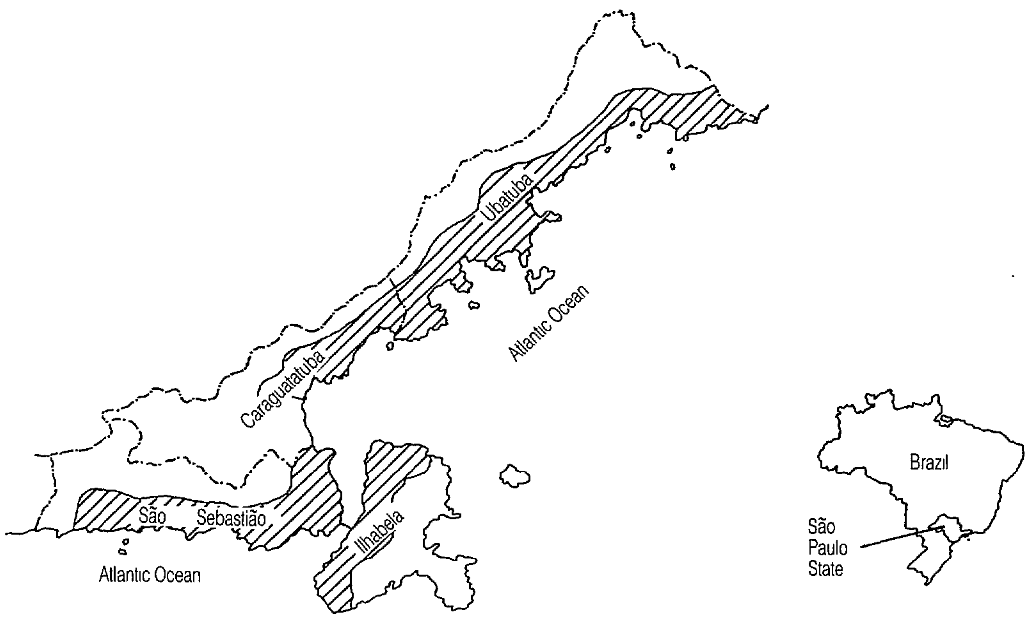
Figure 1. A map showing the survey area along the North Littoral Zone of São Paulo State.



tions are carried out over an area of 893 km² within the region, which is known to contain some 8,094 black fly breeding sites (see Figure 1). The treatment methodology involves application of temephos to target streams on a fortnightly basis. Because of its wide distribution and dense population throughout the area in question, *Simulium pertinax* is considered the main target species (*1*).

The discovery and development of *B. thuringiensis* (H-14) has provided an efficacious and highly selective tool for controlling black fly and mosquito larvae that has minimal impact on aquatic ecosystems (*2, 3*). It has proved especially useful in places where application of conventional chemical insecticides is undesirable for environmental reasons or where development of resistance has reduced the efficacy of chemical larvicides (*4, 5*). In this vein, it is worth noting that the highly complex nature of *B. thuringiensis'* parasporal endotoxin reduces the likelihood of resistance developing in target species. Therefore, in areas such as the North Littoral Zone of São Paulo State that have been receiving regular applications of organophosphorus insecticides to control simuliid larvae, treatment with *B. thuringiensis* (H-14) could prove a safe and effective supplementary or alternative control method.

The purpose of the study reported here was to evaluate the ability of three commercial *B. thuringiensis* (H-14) formulations to control *S. pertinax* under field conditions.

MATERIALS AND METHODS

Between May and September of 1984, three commercial flowable concentrate

(FC) formulations of *B. thuringiensis* (H-14) were tested under field conditions against various *Simulium* species in 24 streams chosen at random in the districts of Ubatuba, Caraguatatuba, and Ilhabela (see Figure 1). The principal target species was *S. pertinax.*

The formulations used and their test sites were as follows: Bactimos FC (Biochem, Solvay Labs, 1,000 International Toxicity Units per milligram) was tested in four streams with discharges varying between 13 and 350 m^3 per minute. Teknar (Zoecon, Sandoz, 1,500 International Toxicity Units per milligram) was tested in seven streams with discharges varying between 5 and 90 m^3 per minute; and Vectobac FC (Abbott Labs, 600 International Toxicity Units per milligram) was tested in 13 streams with discharges varying between 0.3 and 132 m^3 per minute.

Stream discharge rates were determined using a stop watch and a float (an orange) to measure current velocity and a measuring tape and a meter stick to measure width and depth. Several measurements of each stream dimension were averaged to compute the discharge rate. The amount of flowable concentrate needed to produce a concentration of 10 mg of product per liter of stream discharge for one minute was calculated for each stream and weighed out in the field using a torsion balance. Sprinkling cans, calibrated in such a way as to obtain a one-minute application of the diluted flowable concentrate, were used for stream treatments in the manner set forth by Lacey and Undeen (*6*).

To determine the effective carry of *B. thuringiensis* H-14 (the greatest distance downstream from the application point where 80% or greater mortality could be observed—*7*) nylon cords 30 cm long and 1 cm in diameter were used as artificial substrates and anchored in groups of 10 at various places above and below the treatment point. To ensure sufficient colonization by black fly larvae, these artificial substrates were placed in the streams seven days before treatment (*8*). The substrates were also distributed in accordance with stream discharge as indicated in Figure 2, taking into account the known relationship between stream discharge and effective carry (*7*).

In streams with discharges between 0.3 and 2.4 m^3 per minute, the initial substrates were placed 10 meters downstream from the treatment point, and additional substrates were placed at intervals of 25 meters—the first of these being 25 meters downstream from the treatment point and the last being 100 meters downstream. Subsequent tests conducted in this same group of streams placed the first substrates 10 meters downstream from the treatment point and additional substrates at 50 meter intervals—the first of these being 50 meters downstream from the treatment point and the last being 250 meters downstream.

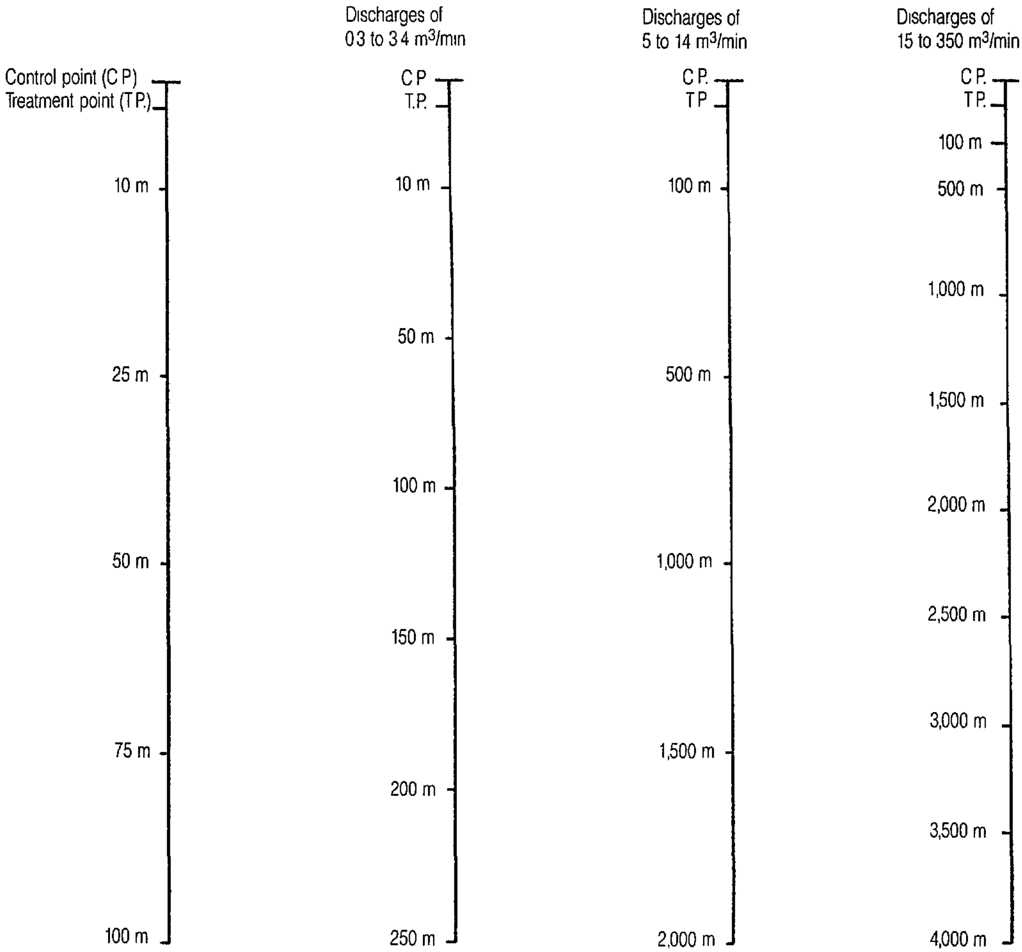
In streams with discharges between 5 and 14 m^3 per minute, substrates were placed 100 meters downstream from the treatment point and also at intervals of 500 meters for 2 km below the treatment point.

In streams with discharges between 15 and 350 m^3 per minute, substrates were placed 100 meters downstream and at intervals of 500 meters for 4 km below the treatment point.

In all of the tests performed, substrates were also placed two or more meters upstream of the treatment point as controls.

Immediately before treating each stream, the stream's discharge rate was determined and five substrates were removed from each sampling point and placed in individual containers holding 70% ethanol. Care was taken not to disturb attached fauna before or during the

Figure 2. A schematic diagram showing the distribution of artificial substrates downstream from breeding sites with different water discharge rates.



removal process. Each container was labeled with the substrates' location and relevant pretreatment and post-treatment information. The number of larvae on each substrate was determined later in the laboratory, and specimens of these larvae were saved for subsequent identification/confirmation. Then, 48 hours after treatment the remaining five substrates at each sampling point were removed and processed like the pretreatment samples. The resulting larval counts made it possible to assess the percentage by which the larvae were reduced at each sampling point.

For purposes of analysis, the streams involved were classified as small, middle-sized, or large on the basis of the stream discharge rate at the test site. Specifically, those streams classified as small had discharge rates of 0.31 to 10 m^3 per minute, those classified as middle-sized had rates of 10 to 45 m^3 per minute, and those classified as large had rates greater than 45 m^3 per minute. For streams with small discharges, the efficacy comparison was made using the t-test for independent samples. In the case of streams with medium and large discharges, the efficacy comparison was made by analyzing variances. Regression analysis was performed on effective carry and discharge data.

RESULTS

In all stream groups, the three products showed similar results in relation to efficacy and effective carry (Table 1). No significant differences were detected among the products at the 0.05 confidence level.

Relationships observed between product efficacy and stream discharge rate are shown in Table 2. Significant correlations were found between stream discharge rates and the effective carry of Bactimos ($R = 0.99$, $p < 0.01$) and Vectobac FC ($R = 0.75$, $p < 0.005$). The absence of a significant correlation between stream discharge rates and the effective carry of Teknar could be related to the narrower discharge range and physical characteristics of the streams selected for its evaluation. Among other things, since the streams were chosen at random, the presence of a considerable number of pools, regions of sluggish flow, or other factors could have had a negative influence on the Teknar formulation's performance.

DISCUSSION AND CONCLUSIONS

The strong correlation between stream discharge and effective carry of *B. thuringiensis* (H-14), also observed by several other investigators (reviewed by Lacey and Undeen—*9*) could create difficulties for control programs in regions with large numbers of very small streams. In order to obtain satisfactory control at breeding sites with low discharge rates, especially when the stream is of considerable length, it may be necessary to apply the product at very short intervals (i.e., at numerous points). This circumstance was previously noted by Undeen et al. (*10*) and Gaugler et al. (*11*) during evaluation of Teknar in small streams in Guatemala and Mexico, respectively, where the effective carry distance was very short.

In places such as São Paulo's North Littoral Zone, where applications are made manually at sites that are commonly hard to reach, the treatment of small breeding sites in any kind of routine control program may prove operationally difficult, and in some cases impossible. However,

Table 1. Efficacy of three commercial formulations of *Bacillus thuringiensis* var. *israelensis* at 10 mg per liter of stream discharge for one-minute applications against *Simulium pertinax* in small streams of São Paulo State's North Littoral Zone.

Formulation	Stream size (m^3/min)	No. of streams tested	Average effective downstream carry (m)[a]
Bactimos	10.0 ⊢— 45	2	100
	≥45	2	1,250
Teknar	0.3 ⊢— 10	3	533
	10.0 ⊢— 45	3	566
	≥45	1	1,000
Vectobac	0.3 ⊢— 10	7	187
	10.0 ⊢— 45	4	525
	≥45	2	1,250

[a] The greatest downstream distance at which 80% larval mortality was observed.

Table 2. Stream discharge rates (m^3/min) and effective carry[a] for *Bacillus thuringiensis* (H-14) at breeding sites of *Simulium pertinax* along São Paulo State's North Littoral Zone.

Treatment formulation used:					
Bactimos		Teknar		Vectobac	
Discharge rate (m^3/min)	Effective carry (m)	Discharge rate (m^3/min)	Effective carry (m)	Discharge rate (m^3/min)	Effective carry (m)
13.0	100	5.22	100	0.30	50
14.6	100	7.70	1,000	0.73	50
45.0	500	8.80	500	1.05	50
347.0	2,000	14.40	1,500	1.05	50
		20.30	1,000	1.10	100
		20.30	1,000	2.10	150
		89.50	1,000	2.40	100
				13.60	100
				28.60	600
				33.00	1,000
				33.10	1,000
				44.40	1,000
				131.50	1,000

[a] The greatest downstream distance at which 80% larval mortality was observed.

in medium and large streams where the effective carry is satisfactory, no operational difficulties would be expected.

As this suggests, the topographic characteristics of the breeding sites greatly influence treatment efficacy. Sluggish or shallow water, or the presence of pools or backwaters, contribute to increased product sedimentation, and hence decreased carry. Indeed, the lack of correlation between discharge rate and effective carry observed during the evaluation of Teknar could have been due to use of such breeding sites in our evaluations. On the other hand, treatment of breeding sites with more homogeneous topography, uninterrupted flow, and greater stream velocity yields greater product efficacy, better carry, and a stronger correlation between discharge rate and effective carry (*9, 12*).

The quantities of *B. thuringiensis* (H-14) products used at the larger breeding sites in our study were excessive relative to the amounts of conventional chemical larvicides required. This could create additional logistic problems during treatment operations at locations where access is difficult. Recently, however, formulations with higher concentrations of active ingredient have been developed (*13*), and such formulations might permit use of smaller dosages than those used in this study.

In general, *Bacillus thuringiensis* (H-14) has proved a useful alternative larvicide for routine control programs where aerial applications are feasible or where streams are readily accessible—due to its satisfactory efficacy at breeding sites in medium and large streams (*14*). However, more advanced studies of application methodology are needed to facilitate routine operations in areas such as São Paulo's North Littoral Zone, where the topography is irregular and access to breeding sites is difficult. In particular, studies should be made of methods that minimize the operational difficulties of treating small breeding sites that arise from the short effective carry at most sites of this kind. Overcoming these difficulties should permit *B. thuringiensis* (H-14) to play an important role in the control of simuliids in mountainous coastal regions with dense tropical vegetation.

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