RESEARCH NOTE

Field Studies with the Bacterial Larvicide INPALBAC for *Simulium* spp. Control in Rio Grande do Sul, Brazil

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Hematophagous insects of the *Simuliidae* family are relevant to public health in the State of Rio Grande do Sul, Brazil, with reports of serious attacks on humans having occurred for many decades, causing intense discomfort, allergic reactions and dermatitis, some cases requiring medical care (MAT Souza 1984 *B Saúde 11*: 8-11).

In 1976 the State Health and Environmental Secretariat (SSMA) launched a series of actions to control this insect, initially using the chemical larvicide Temephos (ABATE 500 E). However, during these activities, research conducted by the Division of Zoonoses and Vectors indicated that the target species, *Simulium (Chirostilbia) pertinax*, was not susceptible to the concentrations of the chemical larvicide that were being used, suggesting possible development of resistance induced by improper use of the product, resulting in turn from improper flow measurement (AL Ruas Neto 1984 *B Saúde 11*: 17-21). Studies along these lines were also carried out in São Paulo by CFS Andrade et al. (1987 Congresso Brasileiro de Entomologia, p. 406, Campinas, SP), clearly indicating that the product had limited efficacy against *Simulium (C.) pertinax* larvae.

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Beginning in 1982, new methodologies were studied for integrated control of this insect (Ruas Neto *loc. cit*., AL Ruas Neto et al. 1985 *B Saúde 12*: 17-20) and efficient forms of flow measurement (RL Silveira 1985 Projeto Simulídeo - IPH/UFRGS, Progress Report no. 7, Porto Alegre, RS, GL Silveira 1997 *Quantificação de Vazão de Pequenas Bacias com Carência de Dados Fluviométricos*, PhD Thesis, IPH/UFRGS, Porto Alegre, 172 pp.). In 1983 the State Simulid Control Program was set up and a methodology was developed for this program using fixed flow meters of the modified "Parshal" type (JF Alfaro 1974 Anais do I Simpósio Internacional de Irrigação, Porto Alegre, RS) in small and medium-sized rural water basins in the State of Rio Grande do Sul, in addition to formulations based on *Bacillus thuringiensis* serovar *israelensis* H 14 (H de Barjac 1978 *CR Acad Sci* Paris *286 D*: 797-800).

After preliminary field tests with two imported formulations of biological larvicides based on *B. thuringiensis* serovar *israelensis* (*B.t.i.*) which proved efficient, biological control began in 1983 (MAT Souza et al. 1994 I Seminário - Qualidade de Águas Continentais no Mercosul, p. 261-278, Porto Alegre, RS, Publication no. 2 of the Brazilian Association of Water Resources) and now covers 135 counties and benefits some 3 million individuals. New formulations based on *B.t.i.* are of interest to the State *Simulium* spp. Control Program, so field tests are being performed in some streams where the program is being implemented, using a formulation developed in Brazil, called INPALBAC (product currently being registered with the Brazilian Ministry of Health), batch T003 B-95, with the purpose of evaluating its efficacy. This insecticide is a concentrated emulsifiable preparation based on an entomotoxic biomass of *B.t.i.*, strain IPS-82, obtained from the Pasteur Institute in Paris and with a production process developed at the Oswaldo Cruz Institute, Rio de Janeiro. The formulation of this biomass enables the product to float on a surface of water for a long time, facilitating its being dragged downstream on creeks and streams.

Five tests were performed in the counties of Barão, Sertão Santana, and Tapejara, State of Rio Grande do Sul, from April 1996 to May 1997. The methodology included placing four white bags of low-density polyethylene as substrate every 100 m in the selected streams and waiting for their colonization by *Simulium* spp. larvae. After having verified the colonization by *Simulium* spp. larvae and pupae, two bags from control points were removed and two bags from each of the other points. The bags were placed in flasks with 70ºGL alcohol and the numbers of larvae and pupae were

counted in the laboratory. Stream water flow was checked at the time of the tests using either a fixed flow meter (chutes of the modified "Parshal" type), constructed previously, or by the method proposed by JW Amrine Jr (1983 *Mosquito News 43*: 17-21) with a float system (ping-pong balls with 25 ml of water as ballast). Based on the observed flow, the product's concentration and carry were calculated using the table of the SSMA Technical and Operational Standards, developed for the Simulid Control Program and reproduced below (Table I), where the amount of the product, in grams, is calculated according to the following formula: Bti (g) = flow (m^3/min) x conc. (ppm). After weighing and diluting, the product was transferred to a 3 l sprinkler and applied all across the stream, from one bank to the other, for 1 min, at the specified points. The tests were read 24 hr after application.

TABLE I

Mobile application scale for *Bacillus thuringiensis* serovar *israelensis* (*B.t.i.*)[a]

Flow (m^3/min)	*B.t.i.* (ppm)	Carry (m)
0 - 0.170	Product not applied	-
0.170 - 0.313	50	50
0.313 - 0.626	50	60
0.626 - 1.260	45	75
1.260 - 2.600	40	125
2.600 - 5.100	30	250
5.100 - 11.000	20	500
11.000 - 16.000	15	750
16.000 - 21.000	12	1000
> 21.000	12	1000

a: based on SSMA Technical and Operational Standards.

Sprinkling of INPALBAC (Table II) on the Canoas and Linha Dobrada streams was performed on different dates, and a variation in water temperature and flow was observed, the latter as a function of the variable rainfall and water runoff on the two different dates, as well as 100% mortality for *Simulium* spp. larvae. The product was always sprinkled at six points, every 125 or 500 m, according to the predicted carry, which was obtained in all instances. During the second test at the Linha Dobrada Stream, the larvicide was only used at three points, since the last three had insufficient infestation. Nevertheless, we observed that the product was active even at points 4 and 5, causing the death of the few larvae colonizing there. Only one test was performed on São Domingos Stream. This stream was so crooked that the points could not be marked at 100 m intervals, we opted to mark just one point, where a 380 m carry was successfully observed.

TABLE II

Application of INPALBAC (*Bacillus thuringiensis* serovar *israelensis*) (*B.t.i.*) against *Simulium* spp. larvae in streams in the State of Rio Grande do Sul, Brazil

Site	Flow (m^3/min)	No. of points applied	Concentration of *B.t.i.* (ppm)	Dose of *B.t.i.* per point (g)	Expected carry (m)	Water temperature (°C)	No. of live larvae at control point		Total no. of live larvae at application points	
							Pre-test	Post-test	Pre-test	Post-test
Barão County, Canoas Stream	8.58	6	20	171.6	500 (obtained)	13	357	215	2241	0
Barão County, Canoas Stream	1.56	6	40	62.4	125 (obtained)	9.5	194	78	2633	0
Sertão Santana County, Linha Dobrada Stream	2.48	6	40	99.2	125 (obtained)	19	104	31	1800	0
Sertão Santana County, Linha Dobrada Stream	1.92	3	40	77	125 (obtained)	15	46	35	250	0
Tapejara County, São Domingos Stream	6.36	1	20	127.2	500[a]	12	620	132	1359	0

a: 380 m observed carry.

In conclusion, the tested formulation based on *B. thuringiensis* serovar *israelensis* INPALBAC proved effective in streams where the State Simulid Control Program is implemented in Rio Grande do Sul, achieving 100% mortality for larvae in the stretches where the product was applied and the expected carry or active transport was achieved, according to the method employed by the SSMA and described in its Technical and Operational Standards. Furthermore, these results were similar to those obtained before when two commercial imported formulations were used in the same streams.