Assessment of *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 aqueous suspension for the control of black fly populations, (*Simulium* spp.) in northern Italy

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Abstract

Four field trials were conducted in Mantua province in northern Italy to evaluate the effect of the *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 based VectoBac® 12 AS larvicide on black flies. Our objective was to examine and compare the efficacy of different concentrations of this microbial larvicide in small plain streams. Larvicidal activity was exhibited in all trials. VectoBac® 12 AS at 2.5 ppm/min rate generated larval mortality from the treatment point to 500 meters downstream. Larvae mortality (over 80%) was observed for 3500 meters downstream of the application site when a concentration of 40 ppm/min was applied.

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Introduction

A large population of black flies (Diptera: Simuliidae) has been reported for a long time in the basin of the river Mincio (northern Italy). This river is called Sarca before entering lake Garda, and from there it flows approximately 65 km past Mantua into the Po river. During spring and summer, there is significant tourist activity in this area. Prior to 2009, particularly in the Sigurtà Park (60 ha near the town of Valeggio sul Mincio, Verona province) and other theme parks, black fly outbreaks have been controlled only with insecticide treatments (Santi *et al*., 2009).

Female black flies are bloodsucking insects that seek blood meals from mammals and birds. Outdoor activities such as gardening and cycling have been severely discouraged due to this host-seeking behavior. In the study area they usually bite cattle and horses; however, bites on humans do occur. Before 2009, large-scale insecticide spray treatments against adults were conducted, with very low efficacy and obvious negative side effects on the environment. After 2009, a biological control project in the Mincio river area against black fly larvae was initiated, and very good efficacy was observed. The program was supported by Peschiera and Valeggio sul Mincio municipalities and private tourism companies. *Simulium paraequinum* Puri (47.05%), *Simulium pseudequinum* Séguy (39.5%) and *Simulium erythrocephalum* (De Geer) (13.45%), were the more common species identified in the study area (Santi *et al*., 2011). The literature related to occurences in Italy reports that only well-oxygenated, fast-flowing water can support large black fly larvae populations (Rivosecchi & Coluzzi, 1962; Rivosecchi, 1978; Ghetti *et al*., 2000; Rivosecchi *et al*., 2007). Eggs are laid in submerged plants, and newly hatched larvae remain on the leaves. In some cases, they have been also found hidden under the edges of rocks. Larvae are filter feeders, which then pupate, and adults emerging from flowing water then restart the cycle. There can be 4 or 5 generations throughout the year. In our study area, black flies overwinter as larvae.

Good control of a black fly outbreak includes the use of larvicides. The use of products based on *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 (*Bti*) against black fly larvae in rivers has been a longtime practice in many countries (Majori *et al*., 1986; Barjac & Sutherland, 1990; Riley & Fusce, 1990; Wegner, 2006; Gray *et al*., 2012). Multiple studies have found no evidence that *Bti* applications affected non-target macroinvertebrates or fish (Molloy, 1992; Jackson *et al*., 1994; McCracken & Matthews, 1997; Jackson *et al*., 2002; Bartninkaitè *et al*., 2006; Bernotiene *et al*., 2008). The aim of

this investigation was to study the relative efficacy of *Bti* on black fly larvae at different rates.

Materials and methods

Three small streams near Goito (Mantua province, Italy - Stream 1, N 45°16'17.52", E 10°42'42.60"; Stream 2, N 45°15'26.09", E 10°43'19.20"; Stream 3, N 45°15'26.09", E 10°43'19.21"); were used in these experiments. Streams 2 and 3 are flowing parallel, separated by a dam. All streams were treated with bacterial larvicide. VectoBac® 12 AS (Valent BioSciences Corp., Libertyville, IL, USA) is a suspension concentrate based on the *Bacillus thuringiensis* subsp. *israelensis* Strain AM65-52 with a potency of 1200 ITU/mg. Velocity, turbidity, and pH of the three streams was determined (Tables 1 and 2). The larvicide was applied from the river banks using 10-L knapsack sprayers. The larvicide was applied at a single point on 19 August and 22 September 2010, and 27 June and 21 November 2011. The calculated product rate was mixed with 1-2 parts of water to guarantee an application time of approximately 10 minutes at concentrations of 2.5, 5 and 40 ppm/min.

Larvae were collected at specific distances downstream of the application site after the larvicide had passed the collection site. After each application, larvae were collected from six different points (only five points during Trial 2 in Stream 2, Trial 3 in Stream 1 and Trial 4 in Stream 1) with their vegetative substrate, and transferred into a 500-mL cup. In our study area, the submerged plants were not evenly distributed along the streams, so it was sometimes difficult to collect large numbers of larvae. After sampling in the streams, the cups were placed in a cold icebox. An untreated control sample was collected in the same manner, but 5-100 meters upstream of the application point. After larval collections, the samples were brought to the laboratory and aerated by aquarium pumps for 24 h. Dead and live larvae were counted 24 h after the *Bti* treatment.

Results

Black fly biology and population in study area

Adults are usually active during early morning, late afternoon and early evening. The mass flight of adults occurred in early April to August in the study area. During the two years of this investigation (2010-2011), it was confirmed that the black flies were overwintering as larvae. Four to five generations were observed. Different larval instars occurred from December to March. *Potamogeton crispus* L., *P. perfoliatus* L., *P. pectinatus* L. and *Myriophyllum spicatum* L. were the more common plant species collected in the breeding site in which larvae were present.

Trial 1

Stream 1 was treated with a low concentration of 2.5 ppm/min. High larval mortality was observed initially, with 88.9% at 150 m, but only 39% after 400 m (Tables 1 and 3). After the treatments, in Streams 2 and 3, very high mortality was observed from the application point to several hundreds meters downstream (Tables 1 and 3).

Trial 2

Larval mortality at the 5 ppm/min concentration can be directly compared in Streams 2 and 3. Very high larval mortality was observed from the beginning of the treated area to several hundred meters downstream (Tables 1 and 4). At 1100 m downstream of the treatment site, larval mortality was 90.4 % and 93.3 % respectively.

Trial 3

In Stream 1, treatment with a concentration of 40 ppm/min produced 100% larval mortality through 1500 m. Only a few larvae were observed alive through Point 4 (2900 m from the treatment point), with 99.1% mortality observed. At 3500 m downstream of the treatment site, 83.6% mortality was observed (Tables 2 and 5). In Stream 3, a concentration of 5 ppm/min produced very high mortality (87.1-93.8%) of black fly larvae through the treatment zone (Tables 2 and 5).

Table 1.Water , stream characteristics and rate of product - Trial 1, 19 August 2010; Trial 2, 22 September 2010.

Date		Temperature (°C)	Turbidity FTU	Ph	Depth average (m)	Width (m)	Flow (m/s)	Discharge (m^3/s)	Rate (L)
19 VIII 2010	Stream 1	22.5	8.8	8.2	0.8	4.6	0.7	2.60	0.40 (2.5 ppm/min)
19 VIII 2010	Stream 2	23.0	8.9	8.3	0.36	2.9	1.31	1.37	0.20 (2.5 ppm/min)
19 VIII 2010	Stream 3	24.0	8.39	8.3	0.52	3.35	1.0	1.70	0.25 (2.5 ppm/min)
22 IX 2010	Stream 2	20	3.73	8.3	0.34	3.8	1.37	1.77	0.50 (5 ppm/min)
22 IX 2010	Stream 3	20	3.73	8.3	0.36	3.8	1.38	1.92	0.50 (5 ppm/min)

Table 2. Water , stream characteristics and rate of product - Trial 3, 27 June 2011; Trial 4, 21 November 2011.

Date		Temperature (°C)	Turbidity FTU	Ph	Depth average (m)	Width (m)	Flow (m/s)	Discharge (m^3/s)	Rate (L)
27 VI 2011	Stream 1	23.0	7.8	8.3	0.67	4.8	0.86	2.77	6.60 (40 ppm/min)
27 VI 2011	Stream 3	22.0	3.65	8.2	0.36	4.2	1.0	1.94	0.58 (5 ppm/min)
21 XI 2011	Stream 1	12.2	3.15	8.5	0.34	3.75	0.73	0.94	2.3 (40 ppm/min)

Table 3. Trial 1 - 19 August 2010 Simuliidae larval mortality treatment with larvicide, rate 2.5 ppm/min.

	Sample distance (m)	No. live larvae	No. dead larvae	Mortality (%)
Stream 1				
Control	0	569	6	1.0
Point 1	50	22	49	69.0
Point 2	100	47	296	86.3
Point 3	150	7	56	88.9
Point 4	200	20	58	74.4
Point 5	300	42	80	65.6
Point 6	400	82	54	39.7
Stream 2				
Control	0	480	7	1.4
Point 1	50	0	38	100
Point 2	100	0	21	100
Point 3	200	0	35	100
Point 4	300	0	13	100
Point 5	400	1	17	94.4
Point 6	500	3	54	94.7
Stream 3				
Control	0	511	8	1.5
Point 1	50	5	47	90.4
Point 2	100	3	38	92.7
Point 3	200	3	280	98.9
Point 4	300	8	30	78.9
Point 5	400	25	73	74.5
Point 6	500	2	6	75.0

Table 4. Trial 2 - 22 September 2010 Simuliidae larval mortality treatment with larvicide, rate 5 ppm/min.

	Sample distance (m)	No. live larvae	No. dead larvae	Mortality (%)
Stream 2				
Control	0	420	2	0.5
Point 1	350	0	72	100
Point 2	550	3	24	88.9
Point 3	750	4	175	97.8
Point 4	900	15	158	91.3
Point 5	1100	17	161	90.4
Stream 3				
Control	0	398	1	0.2
Point 1	200	0	17	100
Point 2	350	0	150	100
Point 3	550	0	26	100
Point 4	750	1	8	88.9
Point 5	900	0	2	100
Point 6	1100	1	14	93.3

Trial 4

The concentration of 40 ppm/min of larvicide generated sufficient *Simulium* larval mortality several thousand meters downstream in Stream 1 (Tables 2 and 6). Larval mortality was observed (89.4%) through Point 5 at 3500 m downstream, even though the water temperature was 12.2°C, as opposed to all other trials in this stream, which had water temperatures of 20.0-23.0°C.

Pooled data

Data from all trials regarding the effects of different concentrations at maximum distances from the treatment points are reported in Figure 1. Only the percent larval mortality at the same sampling distances (Tables 3-6) was analyzed.

Discussion and conclusions

Larvicide applications conducted at the correct timing of the black flies' cycle reduced the population. At known breeding sites, a well-calculated concentration of larvicide released into the water produces larval mortality and reduces adult black fly numbers. Efficacy of *Bti* against black fly larvae in the study area was high. The low concentration of 2.5 ppm/min generated larval black fly mortality several hundred meters downstream of the treatment site. The concentration of 5 and 40 ppm/min of *Bti* generated *Simulium* larval mortality in all of these trials. In according with Gray *et al*. (2012), low water temperature did not affect *Bti* efficacy. Comparing the November trial at a water temperature of 12.2°C with the other trials carried out in summer at 23°C, demonstrated that water temperature had no effect on larvicide performance. In any case, if low mortality at very low water temperatures (below 10°C) can be seen, it is recommended to use a higher product rate, as indicated on the VectoBac® 12 AS label, to obtain black fly larval control.

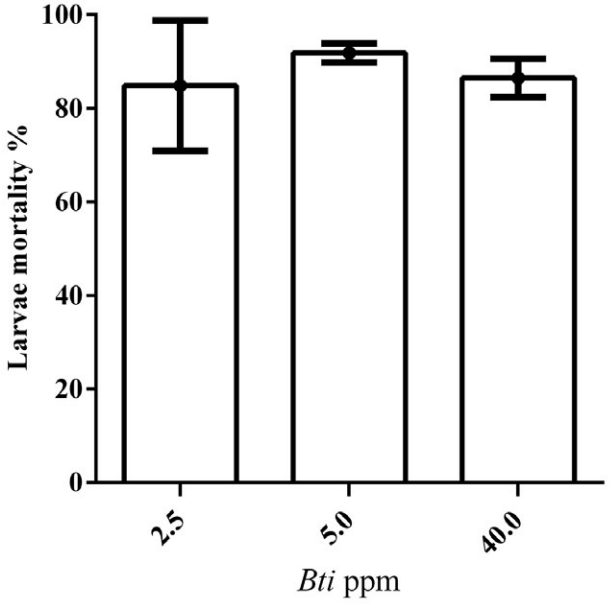

Figure 1. Average±standard deviation (SD) larvae mortality at maximum distance from the treatment point. Rates: 2.5 ppm at 500 m; 5 ppm at 1100 m; 40 ppm at 3500 m.

Table 5. Trial 3 - 27 June 2011 Simuliidae larval mortality treatment with larvicide. Stream 1, rate 40 ppm/min; Stream 3, rate 5 ppm/min.

	Sample distance (m)	No. live larvae	No. dead larvae	Mortality (%)
Stream 1				
Control	0	501	2	0.4
Point 1	650	0	200	100
Point 2	1500	0	155	100
Point 3	2200	1	206	99.5
Point 4	2900	2	218	99.1
Point 5	3500	18	92	83.6
Stream 3				
Control	0	548	2	0.4
Point 1	500	0	17	100
Point 2	800	21	142	87.1
Point 3	1000	0	6	100
Point 4	1400	16	244	93.8
Point 5	1500	26	282	91.6
Point 6	1800	12	183	93.8

Table 6. Trial 4 - 21 November 2011 Simuliidae larval mortality treatment with larvicide, rate 40 ppm/min.

Stream 1	Sample distance (m)	No. live larvae	No. dead larvae	Mortality (%)
Control	0	545	2	0.4
Point 1	650	0	300	100
Point 2	1500	8	522	98.5
Point 3	2500	11	327	96.7
Point 4	2900	7	408	98.3
Point 5	3500	38	321	89.4

The reduction of black fly swarming in the Mincio basin river area after 2009 was significant (Santi *et al.*, 2011). Stakeholders and the general populace have expressed their satisfaction, and also the economic impacts in contrast to other, ineffective pesticide treatments. We hope that, because *Bti* is an effective and environmentally friendly treatment, we can encourage the adoption of this control practice in other river basins where black fly populations still pose a danger, particularly to horses and cows.

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