IMPACT OF THE BLACK FLY (DIPTERA: SIMULIIDAE) CONTROL AGENT *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* ON CHIRONOMIDS (DIPTERA: CHIRONOMIDAE) AND OTHER NONTARGET INSECTS: RESULTS OF TEN FIELD TRIALS

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ABSTRACT. Except for moderate mortality among filter-feeding chironomids, *Rheotanytarsus* spp., the results of 10 field trials with *Bacillus thuringiensis* var. *israelensis* (B.t.i.) indicated a wide margin of safety to the chironomid community and other stream nontarget insects. Mayflies, caddisflies and 2 other types of chironomids, i.e., tube-dwelling (Chironominae) and surface-dwelling, caseless larvae (mix of Chironominae, Diamesinae, Orthocladiinae, Tanypodinae), did not appear to be affected. The susceptibility of filter-feeding chironomids was considerably less than black flies; for example, 4 months of data collected during an operational black fly control program indicated a mean (±95% CI) mortality among *Rheotanytarsus* larvae of 23(15–32)% vs. 98(97–99)% among black flies. Although clearly demonstrating the potential of adverse impact on filter-feeding chironomids in operational black fly programs, these trials also confirmed the narrow impact of *B.t.i.* on the overall stream insect community.

INTRODUCTION

The bacterium *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (*B.t.i.*) is considered to have low nontarget impact when applied to streams and rivers for the control of black fly (Diptera: Simuliidae) larvae (Dejoux and Elouard 1990, Lacey and Mulla 1990, Molloy 1990). Lethality has been clearly demonstrated against only a small group of nontargets, almost all of which are flies in the superfamily Culicoidea (Diptera: Nematocera). Within this superfamily, the family Chironomidae is the most likely nontarget group to suffer some mortality (Car and de Moor 1984, Pistrang and Burger 1984, Back et al. 1985, de Moor and Car 1986).

This paper presents the results of an extensive series of field trials in small streams to further investigate the safety of *B.t.i.* formulations to nontarget insects, with the major focus on chironomids. These trials were conducted to provide the agency that issues permits for black fly control within New York, the New York State Department of Environmental Conservation, with additional data on the impact of *B.t.i.* in stream communities.

MATERIALS AND METHODS

Ten field trials were conducted in northern New York State during April–August in streams with moderate to high densities of black fly larvae. Test streams lacked extensive vegetation and had riffles of cobble/gravel interspaced with silt bottom pools. Test variables included formulation, dosage, number of applications per stream, temperature and stream discharge, as outlined in Table 1. Prior to stream treatments, discharge was measured following the methods of Molloy and Struble (1988). Formulations were applied simply by spreading aqueous suspensions across a stream using a plastic cup. Some field trials required one application, while 480 applications were made in the operational program (field trial 10). Water temperature range during the study was 0–23°C. Field trials 1–9 represented relatively small-scale, short-term (one wk) experiments in low-discharge (320–20,740 liters/min), 1–5 m wide streams. Field trial 10, in contrast, was conducted as part of a 4-month (April-July) operational black fly control program (Molloy and Struble 1989); it involved stream retreatments every 3–4 wk along 32 km of stream length and included streams ranging from rivulets (50 liters/min) to 10 m wide (76,100 liters/min). Depending on existing stream conditions, resources available and degree of quantification desired, a variety of techniques were used to measure the impact on chironomids and other nontargets as outlined below.

FIELD TRIAL 1

Objective: Assessing impact on nontargets by recording change in their densities: Submerged plastic ribbons (2.8 × 30 cm) were used as artificial substrates. Eighty were placed in both an upstream control area and 2,000 m downstream of the treatment point at 4 days pretreatment. Each ribbon was numbered to allow for random removal and was held in place at one end by a 100 g lead weight. Half of these ribbons were removed and placed in alcohol at each sampling area at 1 h pretreatment and the remaining half at 4 days posttreatment. These preserved insects were subsequently identified and their densities (no./m^2) calculated.

Table 1. Treatment parameters relating to the 9 small-scale and one large-scale field trials conducted with *B.t.i.* formulations in small streams.

Field trial no.	Results in table no.	Treatment information	Month of trial	Formulation	Dosage applied	Temp. (°C)	Stream	Discharge (liters/min)
				Small scale field trials				
1	2	Single application	July	Bactimos®WP	3.7 ppm/15 min	12	Camden Creek, Shushan, NY	8,598
2	3	Single application	July	Teknar®WDC	13.4 ppm/15 min	17	White Creek, Cambridge, NY	7,456
3	4	5 applications (every 200 m from 0–800 m)	July	Teknar®WDC	10 ppm/1 min (each application)	15	Negro Brook, Onchiota, NY	20,740
4	4	9 applications (at the following points downstream: 0, 40, 130, 205, 280, 480, 800, 1230 and 1780 m)	June	Teknar®WDC	20 ppm/1 min. (each application)	11	Negro Brook, Onchiota, NY	17,222
5	5	5 applications (every 50 m from 0–200 m)	August	Vectobac®WP	10 ppm/1 min (each application)	17	Murray Hollow Creek, Cambridge, NY	580
6	6	Single application	August	Vectobac®WP	1 ppm/1 min	17	Murray Hollow Creek, Cambridge, NY	211
7	6	Single application	August	Vectobac®WP	50 ppm/1 min	17	Murray Hollow Creek, Cambridge, NY	168
8	6	Single application	August	Teknar®WDC	5 ppm/1 min	17	Murray Hollow Creek, Cambridge, NY	320
9	7	12 applications (every ca. 65 m from 0–790 m)	April	Vectobac®WP	30 ppm/1 min (each application)	3	Negro Brook, Onchiota, NY	10,116
				Large-scale operational field trial				
10	8	480 applications (at variety of intervals of distance depending on discharge)	April through July	Vectobac®WP and Teknar®WDC	Range from 10 to 30 ppm/1 min	Range 0–23	32 km of streams near Onchiota, NY	Range 50–76,100

Table 2. Field trial 1: changes in densities of nontarget insects on artificial substrates following treatment with Bactimos® WP.[a]

Location and time of sampling	Surface-dwelling chironomid larvae		Mayfly nymphs		Caddisfly larvae		Total	
	No./m²	% change	No./m²	% change	No./m²	% change	No./m²	% change
Upstream control								
1 h pretreatment	1,991		368		28		2,387	
4 days posttreatment	6,508	+227	705	+92	61	+118	7,274	+205
2,000 m downstream								
1 h pretreatment	3,025		558		36		3,618	
4 days posttreatment	5,683	+88	835	+50	72	+100	6,590	+82

[a] Change in no./m² of black flies at upstream control and 2,000 m site, respectively, was +70% and −76%.

Table 3. Field trial 2: changes in mean number of nontarget insects in 5 Surber samples following treatment with Teknar® WDC.[a]

Location and time of sampling	Surface-dwelling chironomid larvae		Mayfly nymphs		Caddisfly larvae		Other insects[b]		Total	
	No.	% change	No.	% change	No.	% change	No.	% change	No.	% change
Upstream control										
1 h pretreatment	724		126		54		40		944	
5 days posttreatment	958	+32	123	−2	65	+20	49	+23	1,195	+27
400 m downstream										
1 h pretreatment	151		105		34		19		309	
5 days posttreatment	341	+126	172	+64[c]	69	+103[c]	33	+74	615	+99[c]

[a] Change in mean number of black flies at upstream control and 400 m site, respectively, was +380% and −100%.

[b] Includes stonefly nymphs, larval and adult elmid beetles, empid larvae and tipulid larvae.

[c] Statistically significant (t-test at 5% level).

Objective: Assessing impact on chironomids by recording change in their body size: In addition to the above-mentioned quantitative assessment method, a qualitative method was also used in field trial 1 to determine if any chironomids had been killed. Since earlier instar chironomids are more susceptible to *B.t.i.* intoxication (Ali et al. 1981), the mean larval body size within chironomid populations would increase following *B.t.i.*-induced mortality. Consequently, measurement of larval size, specifically postgenal length on head capsules, was used to determine whether mortality had occurred in the chironomid larval community. Fifty caseless larvae (a mix of Chironominae, Diamesinae, Orthocladiinae, Tanypodinae) were collected with an artist's brush from artificial substrates at 2,000 m downstream of the treatment point immediately pretreatment and again at 4 days posttreatment. As an experimental control, an equal number also was collected concurrently from upstream of the treatment point. Larvae were preserved in 70% alcohol, and their postgenal lengths subsequently measured at the laboratory. Mean sizes at these sites were then compared for statistical significance by t-test ($P < 0.05$).

FIELD TRIAL 2

Objective: Assessing impact on nontargets by recording change in their densities following an intentional overdose: An overdose (equivalent of 201 ppm/1 min applied as a maximum challenge to stream nontargets. Five Surber samples were taken at 1 h pretreatment and at 5 days posttreatment at 400 m downstream of the treatment point and in an upstream control. Each sample was placed in a jar containing 70% alcohol. Insects were subsequently sorted and counted at the laboratory. Mean numbers per sample were compared for statistical significance by t-test ($P < 0.05$).

FIELD TRIALS 3–10

Objective: Assessing impact on chironomids by examination of living and dead larvae: In these 8 field trials, 3 types of late-instar chironomid larvae were checked for mortality both pretreatment and posttreatment; these were caseless surface-dwellers, filter-feeders and tube dwellers. Larvae which did not move after being touched with a probe were considered dead, with magnification (10×) used as needed in this proc-

Table 4. Field trials 3 and 4: mortality among black flies and chironomids present on stream substrates at one day following treatment with Teknar® WDC.

Trial 3			Trial 4			
Sampling sites (m downstream from the initial treatment point)	% mortality (no. sampled)		Sampling sites (m downstream from the initial treatment point)	% mortality (no. sampled)		
	Black flies	Surface-dwelling chironomids		Black flies	Surface-dwelling chironomids	Filter-feeding chironomids
Upstream control	0 (124)	3 (120)	Upstream control	0 (111)	—	9 (75)
200	86 (62)	0 (263)	40	95 (184)	7 (30)	12 (50)
400	100 (65)	3 (150)	130	99 (141)	0 (22)	25 (47)
600	100 (30)	0 (60)	205	100 (119)	0 (44)	41 (32)
800	93 (30)	4 (70)	280	100 (3)	0 (3)	23 (30)
1,000	97 (34)	1 (120)	1,780	100 (103)	—	9 (44)

ess. Caseless larvae were collected from stream substrates (stones, wood debris, vegetation) and were usually immediately examined in the field. Filter-feeding larvae live in cases attached to substrates and were typically found on the underside of rocks. A mortality check of filter-feeding larvae was more time consuming, and they were often field collected and subsequently examined at the laboratory; their cases were either teased apart with forceps or a pencil was rolled slowly forward from the case's apex to force the larva out. Tube-dwelling larvae live within tunnels constructed of fine sand grains and organic matter. To obtain these larvae, bottom sediments were scooped up in a glass jar from stream pools, passed through a 250 μm sieve at the laboratory, and the tubes dissected.

RESULTS

Filter-feeding chironomids (*Rheotanytarsus distinctissimus* gp. and *R. exiguus* gp.), were the only nontarget group to be adversely impacted in the 10 trials (Tables 2–8). Mayflies, caddisflies, and the other 2 types of chironomids, i.e., tube-dwelling (Chironominae) and surface-dwelling, caseless larvae (mix of Chironominae, Diamesinae, Orthocladiinae, Tanypodinae), did not appear to be affected.

In field trial 1, no statistically significant difference was found between the size of chironomid larvae pretreatment vs. 4 days posttreatment at the 2,000 m site: mean (±95% CI) postgenal lengths before and after treatment were, respectively, 171(153–188)μm and 162(143–181)μm. Upstream controls similarly showed no significant change from pre- to posttreatment, i.e., 120(108–132)μm and 140(120–160)μm. These data indicated that surface-dwelling, caseless chironomids were unaffected at the 2,000 m site where black fly densities had been reduced by 76%. Among susceptible insect populations, mortality due to *B.t.i.* ingestion is almost invariably higher among early instars. As a consequence, insect populations, such as black flies, which have been reduced by *B.t.i.* treatments have survivors with a mean body size larger than in the pretreatment population (Molloy and Jamnback 1981). As expected due to the selective elimination of earlier instars, mean black fly larval size (as also measured from postgenal length) significantly increased at the 2,000 m site from a mean (±95% CI) of 236(218–255)μm pretreatment to 322(315–329)μm posttreatment. Further evidence in field trial 1 of a lack of adverse impact on chironomids and other nontargets was the increase in their densities on artificial substrates at 4 days posttreatment (Table 2).

Table 5. Field trial 5: mortality among chironomids present on stream substrates at one day following treatment with Vectobac® WP.[a]

Sampling sites (m downstream from the initial treatment point)	% mortality (±95% CI)[b]		
	Surface-dwelling chironomids	Filter-feeding chironomids	Tube-dwelling chironomids
Upstream controls	0	0	0
40	0	65 (43–84)	0
140	2 (0–10)	91 (64–100)	0
240	0	80 (66–91)	0

[a] 100% black fly mortality at all sampling sites.
[b] Mean of 3 samples where n = 30, 20 and 20, respectively, for surface-dwelling, filter-feeding and tube-dwelling larvae.

Table 6. Field trials 6, 7 and 8: mortality among chironomid larvae present on stream substrates at one day following treatment with Vectobac WP® and Teknar® WDC.[a]

Distance downstream from the treatment point (m)	% mortality (±95% CI)[b]			
	Trial 6 Vectobac® WP	Trial 7 Vectobac® WP		Trial 8 Teknar® WDC
	Filter-feeding chironomids	Surface-dwelling chironomids	Tube-dwelling chironomids	Filter-feeding chironomids
Upstream control	3 (2–4)	4 (0–19)	3 (0–21)	4 (0–23)
15[c]	6 (2–13)	9 (2–18)	8 (1–18)	45 (37–52)

[a] Black fly mortality was 0% at upstream control in all trials and was 94, 100 and 84% at the 15 m sites, respectively, in trials 6, 7 and 8.
[b] Mean of 3 samples where n = 34, 30 and 20, respectively, for trials 6, 7 and 8.
[c] Corrected data presented (Abbott's formula).

Table 7. Field trial 9: mortality among black flies and chironomids present on stream substrates at one day following treatment with Vectobac® WP.

Sampling sites (m downstream from the initial treatment point)	% mortality (number sampled)		
	Black flies	Surface-dwelling chironomids	Filter-feeding chironomids
Upstream control	0 (180)	0 (17)	0 (38)
40	79 (29)	0 (9)	21 (61)
95	100 (44)	0 (5)	38 (21)
125	100 (18)	0 (12)	30 (71)
150	100 (59)	—	32 (111)
200	100 (275)	—	21 (75)
240	100 (138)	0 (25)	6 (16)
315	100 (154)	—	18 (89)
380	100 (183)	—	10 (81)
545	100 (220)	0 (48)	—
565	100 (231)	3 (33)	75 (20)
590	100 (197)	6 (49)	82 (40)
635	99 (176)	0 (8)	61 (18)
680	100 (138)	0 (6)	56 (50)
780	100 (74)	—	60 (81)
880	100 (54)	—	80 (41)
960	100 (68)	—	43 (46)
1,045	99 (127)	—	57 (35)

Table 8. Field trial 10, the 4 month operational program in 32 km of stream length: mortality among black flies and chironomids present on stream substrates at 6–24 h following treatment.

Sampling areas	Black flies			Surface-dwelling chironomids			Filter-feeding chironomids		
	Total no. samples	Total no. larvae examined	Mean % mortality (±95% CI)	Total no. samples	Total no. larvae examined	Mean % mortality (±95% CI)	Total no. samples	Total no. larvae examined	Mean % mortality (±95% CI)
Upstream controls	38	3,465	1 (0–4)	4	85	1 (1–1)	8	1,989	4 (3–5)
Treated areas	131	11,688	98 (97–99)	58	2,851	1 (0–1)	38	2,851	23 (15–32)

No evidence of adverse impact was likewise apparent from Surber sample data in field trial 2. Posttreatment increases in density downstream of the treatment were greater than those recorded in the upstream control for all insect groups (Table 3).

Field trials 3–10 focused exclusively on chironomid larvae (Tables 4–8). While caseless surface-dwellers and tube-dwellers appeared unaffected in all trials, some filter-feeders consistently died following all *B.t.i.* treatments. In general, when black fly mortality was very high (e.g., 91–100%), filter-feeding chironomid mortality was moderate (ca. 9–60%) (Tables 4, 6 and 7). An adverse impact among filter-feeding chironomids was not limited to a particular formulation or temperature (Tables 4–8); mortality occurred when either of 2 formulations was used and across a wide temperature range (i.e., 0–23°C).

DISCUSSION

Chironomids were the most carefully monitored nontarget group in these trials, and the data clearly indicated a wide margin of safety to all except the filter-feeders, *Rheotanytarsus* spp. Almost a decade ago, filter-feeding chironomids were predicted to be the nontarget insect most likely to be affected by *B.t.i.* applications (Gaugler and Finney 1982). *Rheotanytarsus* larvae construct silken nets which filter-out particles drifting downstream; thus, these chironomids, as well as black flies and mosquitoes, have a feeding behavior which serves to concentrate *B.t.i.* from the water column—thereby increasing the likelihood of their intoxication and death. These current trials have provided extensive evidence that the *Rheotanytarsus* spp. inhabiting small streams can be killed at dosages within operational control levels in temperate climates (5–50 ppm/1 min). The mean filter-feeding chironomid mortality during field trial 10 was 23% (Table 8). Judging from the 4 months of data collected in this latter field trial, moderate levels of filter-feeding mortality can be expected in operational black fly control programs. The susceptibility of filter-feeding chironomids, however, was clearly less than black flies, which incurred an average of 98% mortality in field trial 10. These results are in agreement with previous field studies which had reported an adverse impact on *Rheotanytarsus* in Michigan (Merritt et al. 1989) and tanytarsine chironomids in Ivory Coast (Dejoux and Elouard 1990) and South Africa (Car and de Moor 1984, de Moor and Car 1986). Mortality to nonfiltering feeding nematocerans, i.e., blepharocerids and 2 chironomid genera, has also been reported

from a North American study (Back et al. 1985) in which an intentional-overdose of ca. 85 ppm/1 min had been applied. In this latter study, it was theorized that these nonfilter-feeding nematocerans ingested *B.t.i.* crystals from benthic surfaces where the crystals had adhered or settled, and this is a highly probable scenario.

Mortality among nonfilter-feeding chironomids did occur in trial 2—a test designed to assess the affect of *B.t.i.* on nontargets at an extreme overdose (equivalent of 201 ppm/1 min or ca. 10 times higher than that typically used in operational programs). Although no reduction in surface-dwelling, caseless chironomids was recorded at the 400 m site (Table 3), dead larvae were observed on vegetation at 4 h posttreatment at 40 m downstream of the treatment point (% mortality undetermined). This indicated that surface-dwelling, caseless chironomid larvae can be killed close to the source of such an extreme overdose. Similarly, Rutschke and Grunewald (1984) observed dead chironomids in a stream trial using a dosage 17 times higher than normal.

These 10 field trials indicate a very low risk to 2 groups of chironomids, i.e., tube-dwelling and surface-dwelling (nonfilter-feeding) larvae. In the test streams, populations of these latter 2 chironomids were far more abundant than filter-feeders, which typically represented less than 1% of the chironomid community. Thus, the overall impact of *B.t.i.* applications on chironomid communities in the test streams appeared very low.

A variety of assessment techniques were employed in this study and the following comments are offered regarding their value and usefulness.

Looking for changes in a nontarget group's mean body size, as used in field trial 1, was a qualitative method of impact assessment. With appropriate upstream controls, a significant increase in a nontarget population's mean size from pre- to posttreatment indicates that mortality, albeit unquantified, has occurred due to selective elimination of younger instars. While only qualitative, this assessment technique is a very cost-effective one, requiring little labor and equipment, and it is highly recommended for initial nontarget assessments. Having identified a nontarget group with a significant posttreatment increase in mean size, quantitative tests can be subsequently conducted focusing on that nontarget.

Posttreatment counting of attached live and dead insects, as used in field trials 3–10, is an economical, quantitative assessment method. The timing of this assessment technique is critical for most nontargets. The counting must occur after the *B.t.i.* treatment has had an opportunity to inflict mortality, yet before the rotting insects become detached. Since some dead insects usually detach before an assessment is performed, this method typically underestimates true mortality, thereby losing some accuracy. Another inherent problem with this assessment approach is selection of the insects; when examining a substrate for insects, it is easier to be distracted by and count a moving insect as compared with a motionless dead one. This technique, however, is the best quantitative assessment method possible for nontargets which live in attached cases, such as *Rheotanytarsus*. In contrast to black fly larvae which drift off in the current when they are dead, *B.t.i.*-killed *Rheotanytarsus* larvae remain in their cases which are permanently attached to substrates. Measurement of their mortality by direct examination of unpreserved larvae in their cases is thus a very simple and highly accurate procedure which can easily be incorporated into a nontarget assessment program.

Determining pre- and posttreatment densities of benthic insects using benthic samplers, such as the Surber used in field trial 2, is a traditional and an excellent way to quantitatively measure impact in the benthic community. This method, however, requires a relatively large labor input to sort out the nontarget organisms from the sample's debris.

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