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THE RESPONSE OF VAAL RIVER DRIFT AND BENTHOS TO *SIMULIUM* (DIPTERA: NEMATOCERA) CONTROL USING *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* (H–14)

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ABSTRACT

CAR, M. & MOOR, F. C. DE, 1984. The response of Vaal River drift and benthos to *Simulium* (Diptera: Nematocera) control using *Bacillus thuringiensis* var. *israelensis* (H–14). *Onderstepoort Journal of Veterinary Research*, 51, 155–160 (1984)

Two trials to test the efficacy of *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (serotype H–14) against target simuliid and non-target aquatic invertebrates were undertaken in the Vaal River near Warrenton in South Africa. In the 1st trail an application of 1,6 ppm/10 min of *B. thuringiensis* resulted in a significant ($P<0,05$) reduction of simuliid larvae in rapids 70 m below the treatment point 40 hours after its application. Further downstream the larvicide was ineffectual because the low flow of the river (6 m^3/s) allowed the *Bacillus* pores to settle out in calmer stretches.

The 2nd trial was carried out upstream of small rapids with a calculated flow of 0,5 m^3/s at a spore concentration of 2,3 ppm/7 min. The effect of *B. thuringiensis* on the benthic population density and drift activity of the benthos was recorded.

A high mortality of simuliid larvae and Ephemeroptera was observed 35 m below the application point 9 hours after the application of the larvicide. The mortality in Ephemeroptera was partially due to the handling of these animals. Population densities of simuliid larvae in the treated rapids decreased 18 hours after application of the larvicide, but small simuliid larvae showed a numerical increase again after 72 hours, indicating rapid recolonization from drifting larvae. Tanytarsine Chironomidae decreased after the application of *B. thuringiensis*, but most other fauna either increased or did not decrease significantly ($P>0,05$). Within 43 minutes after treatment of the rapids with the larvicide, simuliid drift increased more than sixtyfold, revealing the immediate irritating effect of the product on the target organisms. Drift of other non-target organisms was not noticeably influenced.

Populations of *Simulium adersi* Pomeroy, *S. chutteri* Lewis, *S. hargreavesi* Gibbins, *S. mcmahoni* de Meillon and *S. damnosum s.l.* Theobald were all significantly reduced ($P<0,05$) after treatment of rapids with *B. thuringiensis*, but *S. damnosum s.l.* showed the lowest mortality and appeared less affected by the product than the other species.

INTRODUCTION

Periodic outbreaks of mammalophilic blackflies, mostly *Simulium chutteri* Lewis, along the Vaal River in South Africa have caused mass losses in cattle and, according to farmers, the death of young animals. These complaints resulted in the establishment of a larval control programme using DDT between 1965 and 1967 (Howell & Holmes, 1969). However, aerial spraying with DDT led to the undesirable eradication of most invertebrates and to the subsequent development of slimy algal mats on stones in the section of the river that had been treated. The ecological balance of the river gradually restored itself, but after the disruptive floods of 1974 Simuliidae developed in even greater numbers than had been recorded previously (Begemann, 1980). In 1977, trials to reduce the numbers of Simuliidae by desiccating the aquatic stages through water-level manipulation at the Vaalharts Diversion Weir were carried out; these proved to be effective up to 30 km downstream from the weir (Howell, Begemann, Muir & Louw, 1981).

Chutter (1968) emphasized the importance of Simuliidae in the ecology of the Vaal River and later Moor (1982b) studied the ecology of Simuliidae (particularly *S. chutteri*) in rapids on the Vaal River at the farm Witrand, 11 km downstream from Warrenton. Population fluctuations in blackfly and other fauna were monitored by collecting drift as well as benthic samples between 1978 and 1981. This study revealed that, provided the population density of *S. chutteri* does not exceed a certain level, predators are important in the control of this pest species.

Undeen, Takaoka & Hansen (1981) reported on the successful use of a product[*] containing *Bacillus thuringiensis* Berliner var. *israelensis* de Barjac (serotype H–14) (hereafter referred to as *B.t.i.*), to control blackflies in a small Guatemalan stream. Subsequently the same product was used by Lacey, Escaffre, Philippon, Sékétéli & Guillet (1982) to treat a large river on the Ivory Coast. Encouraged by the results achieved by these workers we chose the same product for our trials to evaluate a method of *Simulium* control where water-level manipulation would not be possible. These trials were carried out in the Vaal River in December 1982. Although *B.t.i.* had been found to have little effect on non-target organisms in a stream in Newfoundland (Colbo & Undeen, 1980), we decided to monitor the response of such organisms, particularly the aquatic predators of Simuliidae, to *B.t.i.*

MATERIALS AND METHODS

Specifications of the product

According to the technical information bulletin supplied, the insecticidal activity of the *B.t.i.* product we used is 1 500 AAU/mg.

No spore density was given but J. G. Gaenssler (personal communication, 1983) informed us that, according to the manufacturers, this product contains $1,6 \times 10^7$ spores/mg. This would give $1,8 \times 10^{10}$ spores/mℓ at a density of 1,14 g/mℓ at 18 °C. To check whether the actual number of active spores corresponded with this claim, spore counts were undertaken from laboratory cultures at Onderstepoort. This enabled us to determine the spore concentration to be applied in our trials. Two replicates of viable colony counts, undertaken on 28 December 1982, resulted in a mean count of $8,2 \times 10^9$ spores/mℓ. A 2nd count 28 days later resulted in a mean of $6,0 \times 10^9$ spores/mℓ. Thus the spore counts for the product, which had been stored at 4 °C since its arrival on 1 December 1982, were lower than those given in the specifications. Judging by the spore counts on 28 December, the actual spore concentrations in the Vaal River after application of the product at 1,6 ppm/10 min was $1,3 \times 10^4$ spores/mℓ.

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[*] Sandoz 402 ISC ('Teknar'), Hoechst Pharmaceuticals (Pty) Ltd

Description of sampling area and methods

The Vaal River from the Vaalharts Diversion Weir downstream to Windsorton flows over a hard stony substrate and drops rapidly in altitude (Chutter, 1968). During the present survey the flow of the river was 6 m^3/s. The water was clear, with a pH of 7,3 (measured with Merck pH paper) and a conductivity of 65 mS/m. Temperatures in the flowing water in rapids on the farm Witrand varied from 23,8 °C at 09h00 on the 7th December 1982 to 27,6 °C at 16h37 on the 9th.

An 11 km stretch of the Vaal River between the Margaretha Prinsloo Bridge at Warrenton and some rapids on the farm Witrand was selected as the treatment area. Sampling points were chosen at 4 rapids, each of which was separated from the preceding rapids by a slower-flowing stretch of the river, as follows: Sampling point I, 70 m below the treatment point at the Margaretha Prinsloo Bridge; sampling point II, 1 km downstream from point I; sampling point III, a further 3,5 km downstream at rapids on the farm Sydney's Hope, and sampling point IV, 6 km below point III at rapids on the farm Witrand.

At each sampling point 5 stones were randomly collected and the fauna on each stone was scraped off into a hand-net with a 92 μm mesh. Fauna collected from each stone was then separately preserved in formalin for later analysis in the laboratory. The surface area of each stone was also calculated, following Moor (1982a). In the laboratory, faunal samples were separated into 2 batches, one retained by a 710 μm mesh sieve and another which had passed through the sieve but was held back by a net with a 92 μm mesh. The animals retained by the sieve were identified as precisely as possible, and counted. Those in the net were identified only to family level before counting. Faunal counts from each stone were expressed as numbers per 1000 cm^2 of stone surface. To determine whether statistical differences occurred between samples collected before and after the application of *B.t.i.*, individual counts of fauna from samples of 5 stones were compared, using the non-parametric Mann-Whitney U-test (Elliott, 1977). To determine whether an overall numerical increase or decrease in faunal numbers had occurred after the application of *B.t.i.*, the converted counts (Nos./1000 cm^2) for each sample from 5 stones were summed and the difference in density of animals per 5000 cm^2 was then expressed as a positive or negative percentage change.

In the 1st trial *B.t.i.* was applied at the Margaretha Prinsloo Bridge at 19h00 on the 7th December 1982 at the recommended concentration of 1,6 ppm/10 min. The *B.t.i.*concentrate was diluted fivefold with water before application with a 10 ℓ watering can. By crossing the river 8 times during the 10-minute treatment period, an assumed random distribution of the larvicide was obtained. The benthic faunal population was assessed before, and 40 hours after, treatment at sampling point I. A visual mortality assessment was undertaken 14 hours after treatment at sampling point I and 15 hours after treatment at sampling point II.

Owing to the slow flow of the water in the river (6 m^3/s) this initial trial was only partly successful. A 2nd trial was therefore undertaken 35 m upstream from sampling point IV in rapids which were separated from the main river channel by a small island. By measuring the water velocity at the foot of the rapids, both near the surface and at the bottom at 0,5 m intervals, a discharge of 0,5 m^3/s was calculated. The larvicide was applied in the same way as in the 1st trial at 13h00 on the 9th December, but at a concentration of 2,3 ppm/7 min.

Stone samples were collected before the treatment, and 18 and 72 hours after treatment. Benthic fauna was also collected 8 hours after the application of *B.t.i.* at a site 35 m below the application point to assess mortality in target and non-target invertebrates. The fauna was immediately examined under a microscope in the field and live, moving, and dead, immobile, animals were separated and preserved for subsequent identification.

For collection of faunal drift in the river, 2 water-wheel drift samplers (Pearson & Kramer, 1969), as modified and used by Chutter (1975) and Moor (1982b), were placed 35 m upstream and immediately below the selected rapids at Witrand. These samplers were calibrated, by measuring the amount of water filtered during 5 revolutions of the water-wheel, before a series of drift samples was collected. Drift filtered through nets with a 92 μm mesh was collected at approximately 2-hour intervals in the afternoon of 8 December before the application of *B.t.i.* and again after its application on 9 December 1982. A further sample was collected between 18h00 on 9 December and 05h00 on 10 December; thereafter samples were taken at 2-hour intervals during the rest of that morning.

The drift samples were enumerated and the results are presented as numbers per 1000 litres.

TABLE 1 Comparison of the benthic fauna in the Vaal River at sampling point I before, and 40 hours after, the application of the Sandoz 402 formulation of *B.t.i.* at the Margaretha Prinsloo Bridge. Invertebrates collected from 5 stones expressed as numbers per 5000 cm^2. Mann-Whitney U-test used for statistical evaluation

Invertebrates	Before treatment	After treatment	Percentage difference	Significance of difference (P=0,05)
Simuliidae:				
Simulium damnosum s.l.	147	98	− 33	NS
S. adersi	59	11	− 81	S−
Small Simuliidae	169	54	− 68	NS
Chironomidae:				
Orthocladinae Type d.h.c.	48	71	+ 48	NS
Orthocladinae Type C	379	483	+ 28	NS
Small Chironomidae	294	198	− 33	NS
Ephemeroptera	31	69	+123	S+
Plecoptera	0	2	—	NS
Trichoptera:				
Cheumatopsyche thomasseti	49	67	+ 37	NS
Amphipsyche scottae	9	16	+ 78	NS
Gastropoda:				
Burnupia sp.	145	61	− 58	S−
Platyhelminthes:				
Planaria sp.	2	7	+350	NS

NS = not significantly different; S− = significantly less; S+ = significantly more

RESULTS AND DISCUSSION

Treatment of the entire stretch of river from the Margaretha Prinsloo Bridge to the farm Witrand

An inspection of stones at sampling point I 14 hours after treatment revealed very few living and no dead simuliid larvae. A reduction in the population of Simuliidae on trailing vegetation was also noticed. A few dead larvae were found attached to sedges in the river.

A benthic faunal population assessment (Table 1) 40 hours after the application of *B.t.i.* showed a significant reduction ($P<0{,}05$) of *Simulium adersi* Pomeroy. The reduction of *Simulium damnosum s. l.* Theobald and small simuliid larvae was not significant ($P>0{,}05$). Non-target faunal groups were not significantly reduced ($P>0{,}05$), except that molluscs of the genus *Burnupia* decreased significantly in numbers. As *Burnupia* spp. are algal browsers, not filter feeders, this decrease was unexpected. Total numbers of Ephemeroptera and Trichoptera increased significantly ($P<0{,}05$), probably because a greater colonizable area became available on stones after the Simuliidae had drifted off. Plecoptera were found only sporadically in the Vaal River during this survey and were therefore not considered in this trial.

At sampling point II all the simuliid larvae appeared to be alive 15 hours after treatment. As the river between sampling points I and II was wide and flowing slowly at 6 m^3/s, *B.t.i.* had probably settled out in that area. It would threfore not have influenced the 2nd trial, which was carried out 11 km further downstream.

Treatment of small rapids at Witrand

(a) *Results of field mortality assessment*

The fauna collected from stones and in drift samples revealed that the effect of *B.t.i.* on the larval simuliid population was significant ($P=0{,}05$). Nine hours after treatment of the river with *B.t.i.*, large numbers of invertebrates in the rapids 35 m below the treatment point had died (Table 2). More than 50 % of all simuliid larvae were killed. Ephemeroptera were affected after this initial period, but the high mortality (44 %) could possibly have been caused by physical damage to the nymphs when they were washed off the stones when samples were taken. Gradual depletion of oxygen levels in the sorting trays may also have caused some of the animals to die. It would, however, be unlikely that the simuliid species were adversely affected by storage in stationary water during the short period when the animals were sorted (as observed by Moor, 1982a; Car, 1983). The pale areas visible where the midgut had dissolved (Fig. 1 & 2) in simuliid larvae were good indicators of mortality caused by *B.t.i.*

In heavily infested simuliid larvae the intestines appeared to be completely dissolved. The larvae were straight, which indicated that the body muscle tonus had been affected (Fig. 1).

(b) *Population estimates of benthic fauna before and 18 and 72 hours after application of* B.t.i.

Simuliid larvae of all 5 species encountered were significantly less abundant both 18 and 72 hours after application of *B.t.i.* (Table 3). It was noticeable, however, that there was a significant increase ($P<0{,}05$) in small simuliid larvae 72 hours after application of *B.t.i.* when

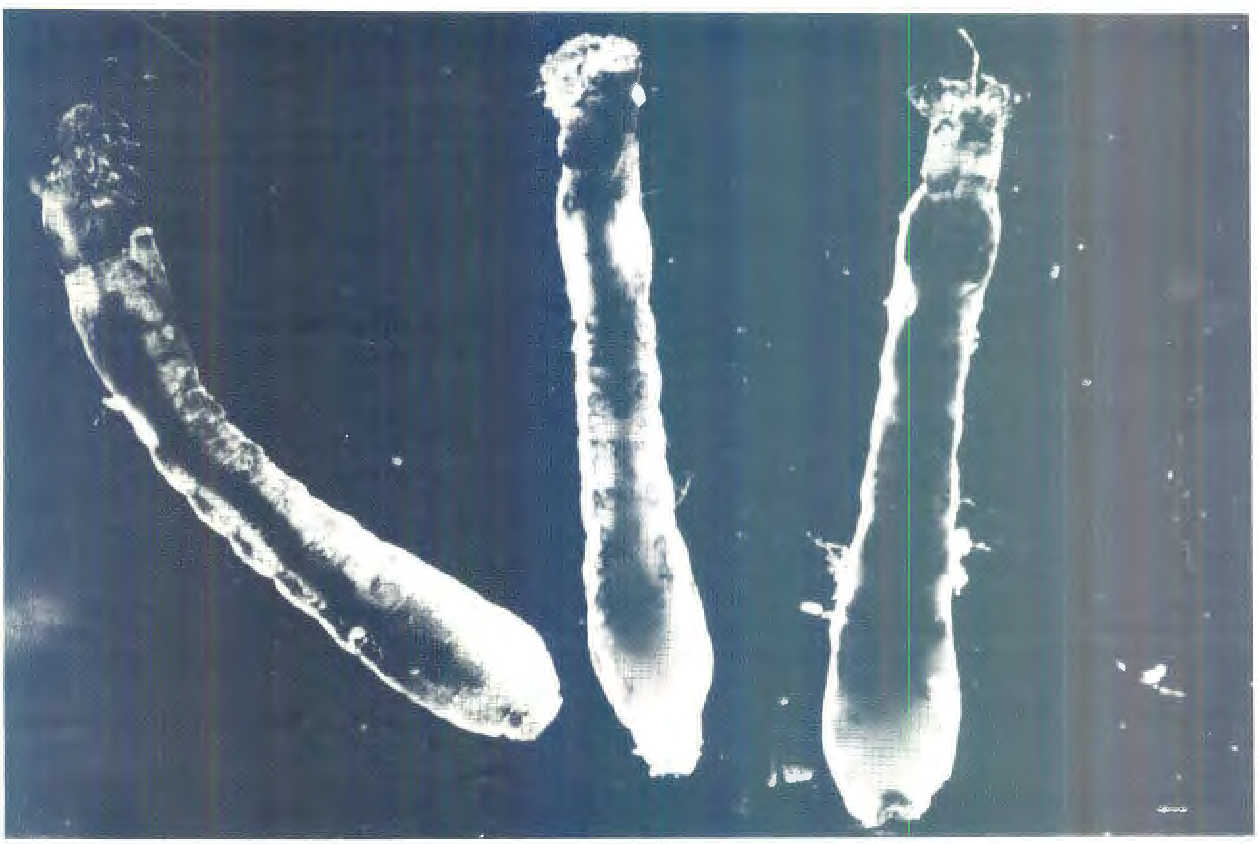

FIG. 1 The effect of *B.t.i.* on *Simulium* spp. larvae
From left to right: First larva unaffected and showing complete gut and good muscle tonus; 2nd larva showing ruptured midgut and no muscle tonus; 3rd larva showing massive gut destruction and no muscle tonus

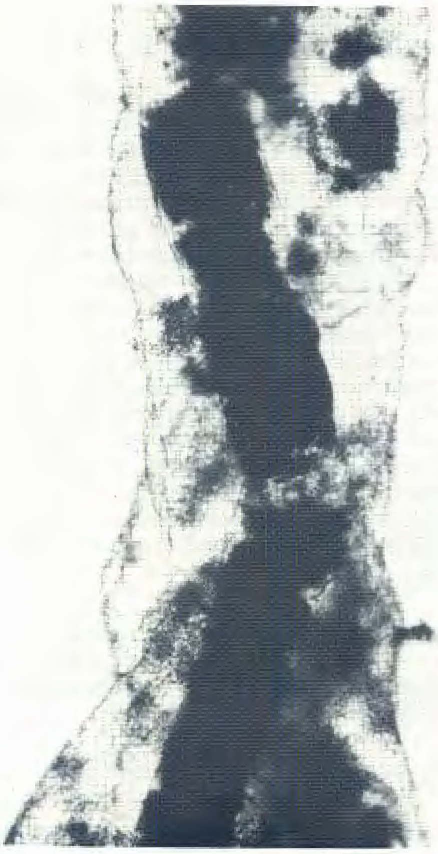

FIG. 2 The effect of *B.t.i.* on the midgut of a *Simulium aderst* larva showing broken-up midgut

compared with the numbers of small larvae 18 hours after application. This indicated that recruitment of larvae from regions upstream from the rapids was noticeable within 72 hours.

None of the observed species of Trichoptera and Ephemeroptera were significantly reduced 18 and 72 hours after the application of *B.t.i.* An actual increase in small hydropsychid larvae, *Baetis glaucus* Agnew and *Centroptilum medium* Crass, was observed after 18 hours, and this increase was still discernible in small hydropsychid larvae after 72 hours (Table 3). This would again indicate that colonization by these animals increased after the application of *B.t.i.*, as noted by Gaugler & Finney (1982). This increase possibly took place because more sites became available for colonization by these animals after the removal of the Simuliidae. A significant decrease in the numbers of *B. glaucus* occurred between 18 and 72 hours after the application of *B.t.i.* This would point to rapid colonization by this species initially, but disturbance by other colonizers (perhaps small Simuliidae) may have led to the overall decrease in numbers of this species.

A significant decrease in Tanytarsini 18 and 72 hours after application of *B.t.i.* would indicate that this group of Chironomidae (the species could not be identified) may also be affected by *B.t.i.* in concentrations effective against Simuliidae.

Burnupia sp. numbers were significantly lower after 18 hours, but not after 72 hours. It may be that patchiness in distribution was responsible for this, as it seems unlikely that such a slow-moving animal could recolonize substrates after 72 hours.

(c) *Faunal drift in the river before and after treatment with* B.t.i.

Faunal drift in the river both above and below the rapids at Witrand was of the same magnitude before treatment of the river with *B.t.i.* (Table 4). Forty-three minutes after the application of *B.t.i.* drift of simuliid larvae off the treated rapids had increased enormously. The intestines of these drifting larvae were not obviously affected at this early stage, so it appeared that *B.t.i.* caused some early irritation leading to the increased drift of Simuliidae. During the next few hours the larval simuliid drift gradually decreased and by 18h45 it was already $^1/_5$th of what it had been 43 minutes after application of *B.t.i.* By the following morning an overnight sample of drift, and drift samples collected during the morning, revealed that the simuliid larvae were once more found in numbers comparable with those encountered prior to the *B.t.i.* treatment. Faunal drift above the rapids and treatment point remained low: it was comparable to drift below the treated rapids before application of *B.t.i.* throughout the time samples were being collected.

Three days after treatment with *B.t.i.* dead simuliid larvae were still found attached to stones, so the drift of larvae off the treated rapids probably consisted of large numbers of dead larvae on 10 December 1982. That the drift of live larvae off the treated rapids would then be lower would be a natural consequence of the recolonization of the rapids by larvae from upstream untreated areas.

At the concentrations used in the trial, the influence of *B.t.i.* on non-target fauna in the drift was negligible.

TABLE 2 Estimated mortality of benthic fauna in the Vaal River at Witrand, 9 hours after the application of the Sandoz 402 formulation of *B.t.i.* (2,3 ppm/7 min), 35 m below the treatment point

Taxon	Numbers of invertebrates			Estimated percentage mortality
	Alive	Dead	Total	
Simuliidae:				
S. adersi	0	3	3	100
S. damnosum s.l.	1	1	2	50
S. hargreavesi	24	138	162	85
Small Simuliidae	7	10	17	59
Chironomidae:				
Orthocladinae Type d.h.c.	25	5	30	17
Orthocladinae Type C	220	10	230	4
Ephemeroptera	9	7	16	44
Trichoptera:				
Cheumatopsyche thomasseti	8	3	11	27
Gastropoda:				
Burnupia sp.	2	0	2	0
Platyhelminthes:				
Planaria sp.	3	0	3	0

TABLE 3 The benthic fauna in the Vaal River at Witrand collected from 5 stones expressed as numbers per 5000 cm^2. Samples taken: I, on December 7 before treatment with the Sandoz 402 ISC formulation of *B.t.i.;* II on December 10, 18 hours after treatment, and III on December 12, 72 hours after treatment. Statistical comparisons were made using the Mann-Whitney U-test

Invertebrates	Total number in samples			Significance (P=0,05)*		
	I	II	III	Sample I compared with Sample II; Sample III		Sample II compared with Sample III
Simuliidae:						
S. adersi	41	0	0	S−	S−	NS
S. chutteri	55	0	0	S−	S−	NS
S. damnosum s.l.	68	1	6	S−	S−	NS
S. hargreavesi	528	15	0	S−	S−	NS
S. mcmahoni	33	2	0	S−	S−	NS
Small Simuliidae	686	97	188	S−	S−	S+
Chironomidae:						
Orthocladinae Type d.h.c.	117	117	156	NS	NS	NS
Orthocladine Type C	540	555	730	NS	NS	NS
Pentaneura sp.	3	0	0	NS	NS	NS
Tanytarsini	74	24	39	S−	S−	NS
Small Chironomidae	1 161	2 914	1 997	NS	NS	NS
Ephemeroptera:						
Afronurus sp.	4	0	0	NS	NS	NS
Austrocaenis sp.	3	0	0	NS	NS	NS
Baetis glaucus	0	16	4	S+	NS	S−
Centroptilum medium	6	34	34	S+	NS	NS
Pseudocloeon maculosum	2	4	3	NS	NS	NS
Tricorythus sp.	0	2	0	NS	NS	NS
Small Baetidae	263	454	437	NS	NS	NS
Trichoptera:						
Amphipsyche scottae	7	16	8	NS	NS	NS
Cheumatopsyche thomasseti	101	119	58	NS	NS	NS
Small Hydropsychidae	0	59	41	S+	S+	S+
Catoxyethira sp.	0	2	0	NS	NS	NS
Orthotrichia sp.	2	3	0	NS	NS	NS
Coleoptera						
Elmidae	0	0	3	NS	NS	NS
Aulonogyrus sp.	0	0	3	NS	NS	NS
Mites	0	27	0	S+	NS	S−
Gastropoda:						
Burnupia sp.	294	46	243	S−	NS	NS
Corbicula sp.	0	6	0	NS	NS	NS
Oligochaeta	0	7	0	NS	NS	NS
Platyhelminthes:						
Planaria sp.	41	78	43	NS	NS	NS

* NS = not significantly different; S− significantly less; S+ significantly more

TABLE 4 Invertebrate drift expressed as numbers per 1000 ℓ water 35 m above and immediately below rapids at Witrand in the Vaal River. Samples collected from 8–10 December 1982. Treatment with the Sandoz 402 formulation of *B.t.i.* (2,3 ppm/7 min) undertaken at 13h00 on December 9, 1982 above the rapids

Drift 35 m above rapids

Date	December 8		December 9				December 10		
Sample collected	17h30	18h52	—	—	—	18h28	05h15	07h13	10h14
Duration (min)	64	74	—	—	—	110	638	106	176
Volume sampled (ℓ)	2 091	2 414	—	—	—	3 584	18 834	3 290	7 629
Simuliidae	1,4	0,8	—	—	—	2,2	1,2	3,9	0,5
Chironomidae	11,3	14,1	—	—	—	9,5	13,4	5,1	7,8
Ephemeroptera	—	0,8	—	—	—	0,5	2,0	0,9	0,3
Trichoptera	—	—	—	—	—	—	0,1	—	—

Drift immediately below rapids

Date	December 8		December 9				December 10		
Sample collected	18h10	19h18	12h33	13h43	16h17	18h45	04h57	06h58	10h30
Duration (min)	58	60	98	64	90	125	600	115	180
Volume sampled (ℓ)	792	725	680	428	1 088	1 310	6 830	1 389	3 050
Simuliidae	11,4	5,5	10,3	659,4	264,8	121,6	5,6	1,4	2,9
Chironomidae	32,8	13,8	10,3	14,0	21,9	23,6	1,7	12,8	11,2
Ephemeroptera	2,5	—	4,4	2,4	0,9	—	2,4	2,8	1,6
Trichoptera	1,3	—	1,5	—	0,9	—	0,1	0,7	0,9

CONCLUSIONS

Previous assessment of *B.t.i.* on non-target insects in the field were undertaken by Colbo & Undeen (1980) and by Molloy & Jamnback (1981). Colbo & Undeen (1980) recorded a significant reduction (P<0,05) of *Hydropsyche sparna* Ross and of Baetidae. Molloy & Jamnback (1981), however, noted large increases in all non-target insects, which they considered was due to inaccurate sampling with Surber samplers. In the present study significant increases of Trichoptera in the 1st trial, and of certain Ephemeroptera in both trials, were based on an analysis of replicate samples and were therefore unlikely to be due to sampling inaccuracy. The increases of non-target benthic insects must have been due to the colonization of vacated habitats created when simuliid larvae drifted off.

Spore counts in the *B.t.i.* product used were estimated to be less than half of those claimed by the company, i.e. $8,2 \times 10^9$ as opposed to $1,8 \times 10^{10}$. According to J. G. Gaenssler (personal communication, 1983) batches of the product leave the factory with higher numbers of spores than necessary for the required insecticidal activity. This is done to ensure that a sufficiently high concentration of spores is maintained over the stipulated shelf-life of the product. The spore concentrations of *B.t.i.* used in the Vaal River trials were within the range of spore concenrations found in previous trials with other products (Gaugler & Finney, 1982).

The influence of *B.t.i.* on aquatic fauna was assessed in 3 ways:

(i) By determining direct faunal mortality;
(ii) by testing faunal population changes on stones; and
(iii) by taking faunal drift samples above and below treated rapids.

The direct assessment of mortality due to *B.t.i.* was the least reliable method, as it was influenced by artificial factors encountered by the invertebrates after their removal from the river. In both trials population estimates revealed that the product was very effective against simuliid larvae, but with the species *S. damnosum s.l.* being the least affected. Account must be taken of the fact that, according to chromosome studies (Car, unpublished data, 1982), *S. damnosum s.l.* in the Vaal River is probably a sibling species hitherto undescribed in Africa.

The benthic population density of non-target predators, such as *Cheumatopsyche thomasseti* (Ulmer) and *Amphipsyche scottae* Kimmins, was not significantly reduced after the application of *B.t.i.*, an indication that it has very little effect on these invertebrates at the concentrations used.

The influence of *B.t.i.* on drift had previously only been studied by Dejoux in troughs set in a stream. His data are unpublished and, according to Gaugler & Finney (1982), in Dejoux's trial the drift rate of non-target insects did not increase. The present study is in agreement with Dejoux's results and revealed that faunal drift is a good indicator for testing the efficacy of *B.t.i.* against simuliid larvae. The unusually low numbers of *S. chutteri*, the actual target species against which *B.t.i.* was to be tested in South Africa, necessitate further trials in areas where this species is abundant, before the product can be considered for regular use in this country.

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