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A FIELD EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AS A BIOLOGICAL CONTROL AGENT FOR *SIMULIUM CHUTTERI* (DIPTERA: NEMATOCERA) IN THE MIDDLE ORANGE RIVER

F. C. DE MOOR[1] and M. CAR[2]

ABSTRACT

MOOR, F. C. DE & CAR, M., 1986. A field evaluation of *Bacillus thuringiensis* var. *israelensis* as a biological control agent for *Simulium chutteri* (Diptera: Nematocera) in the Middle Orange River. *Onderstepoort Journal of Veterinary Research*, 53, 43–50 (1986).

Bacillus thuringiensis Berliner var. *israelensis* de Barjac (Serotype H-14) (*B.t.i.*) at a concentration of 1,6 ppm/10 min and a toxicity of 1500 AAU/mg was tested against *Simulium chutteri* Lewis larvae in the Orange River near Prieska, South Africa. Samples of benthic fauna from the stones-in-current biotope were collected before application of the product and at various intervals up to 80 h afterwards at 4 stations from 200 m to 11 km downstream of the application site. Faunal drift increased slightly after the arrival of the *Bacillus* at 2 stations 1,4 and 6 km respectively downstream of the application site.

Large numerical decreases in benthic simuliid larval numbers after the application of *B.t.i.* in the Orange River were not statistically different ($P>0,05$). This indicated that the size of replicated samples that showed significant decreases ($P<0,05$) of simuliid numbers in the Vaal River was not adequate to show statistical differences in the Orange River. The quantity of dead larvae on stones collected from rapids after application of the *B.t.i.*, and the numerical decreases found by comparing median values of larval counts on stones indicated that *B.t.i.* effectively killed simuliid larvae. Three days after application of the *Bacillus*, recruitment of small simuliid larvae on stones 1,4 km downstream of the application site was discernible again. Tanytarsini were also numerically reduced after *B.t.i.* application. At a flow rate of 38 m^3/s *B.t.i.* was visibly effective in killing *S. chutteri* up to 6 km downstream of the application site and statistically significant decreases ($P<0,05$) in numbers of larvae were seen at a site 11 km downstream of the application site.

The use of *B.t.i.* in *Simulium* control is preferable to organophosphate and organochloride formulations because it has a more specific action against blackfly and because there is no known immunity to *B.t.i.* in any *Simulium* species. However, we must advise that *B.t.i.* should be screened against all co-existing fauna in each situation where new community structures of animals are encountered. Several methods for improving the efficacy of *B.t.i.* are suggested.

INTRODUCTION

Pest outbreaks of the mammalophilic blackfly *Simulium chutteri* Lewis along the Vaal and Orange Rivers have been controlled by 3 strategies. The first was the use of a broad spectrum pesticide (DDT) applied by aircraft either over a selected stretch of river or directly into the river below an impoundment (Howell & Holmes, 1969). Secondly, manipulation of the river flow, downstream of an impoundment, was used to disrupt the aquatic stages of Simuliidae by exposing and desiccating the sessile pupae and causing the larvae to drift into pools downstream of rapids (Howell, Begemann, Muir & Louw, 1981). The 3rd method involved an integrated form of control whereby data on the life-cycle and biology of *S. chutteri* and its natural aquatic invertebrate predators were used to determine the best time to carry out a series of river-flow cessations. Water flow regulation was then applied to check the build-up of *S. chutteri* populations and maintain them at levels at which control by natural predators sufficed to prevent outbreaks of pest proportional size (Moor, 1982 a & b).

Integrated water flow regulation, the cheapest and ecologically the least disruptive of the above 3 methods of *Simulium* control, is however limited by the availability of impoundments upstream of *Simulium* breeding sites in a river. Because of their ecologically disruptive effects, broad spectrum pesticides are preferably avoided. The recent successful use of a highly specific delta-endotoxin, produced by *Bacillus thuringiensis* var. *israelensis* (serotype H-14) de Barjac (hereafter referred to as *B.t.i.*) and prepared as an emulsifiable powder or liquid, against Simuliidae in South Africa provides a 4th method for blackfly control, especially useful in regions of rivers where regulation of the water flow is not possible (Car, 1984; Car & Moor, 1984).

In December 1982, trials in the Vaal River proved that *B.t.i.* killed *Simulium* larvae for a limited distance downstream of the application point (Car & Moor, 1984). The low flow of the river (6 m^3/s) and the extensive pools below the rapids limited the downstream carry, and it became clear that the effectiveness of *B.t.i.* in a large South African river remained to be evaluated. As the flow rates in the Orange River are considerably higher than those in the Vaal River, and as the most serious problem blackfly, *S. chutteri*, occurs in large numbers in this river (Car, 1983), we chose this river for the evaluation of carry and efficacy of *B.t.i.*

MATERIALS AND METHODS

Specifications of the product used

The *B.t.i.* containing product, Sandoz 402 ISC (TEKNAR), was provided by Hoechst Pharmaceuticals (Pty) Ltd, South Africa. Spore counts from laboratory cultures at Onderstepoort revealed a mean of $16,0 \times 10^9$ spores/$m\ell$ (2 replicates of viable colony counts). However, the quantity of delta-endotoxin found in parasporal bodies of *B.t.i.* bears no direct relationship to the number of spores. The toxicity of a particular strain or formulation of *B.t.i.* is, therefore, usually expressed as the insecticidal activity of that particular strain when compared to a standardized spore-crystal preparation (Institut Pasteur Standard "IPS 78" or "IPS 80"). The toxicity is expressed either as International Toxic Units for *Aedes aegypti* larvae (ITU/mg) or as *Aedes aegypti* units (AAU/mg). The *B.t.i.* product used in this evaluation was 1500 AAU/mg.

Description of the sampling area

The section of the Orange River selected for the field trials was *c.* 340 km downstream of Lake le Roux. Acocks (1975) classified the surrounding vegetation of this area as Orange River broken veld. The area lies on

[1] National Institute for Water Research, Council for Scientific and Industrial Research, Pretoria 0001. Present address: Albany Museum, Somerset Street, Grahamstown 6140, where requests for reprints should be made

[2] Veterinary Research Institute, Onderstepoort 0110. Present address: Ad. Hruzastr. 3, A-2345 Brunn am Gebirge, Austria

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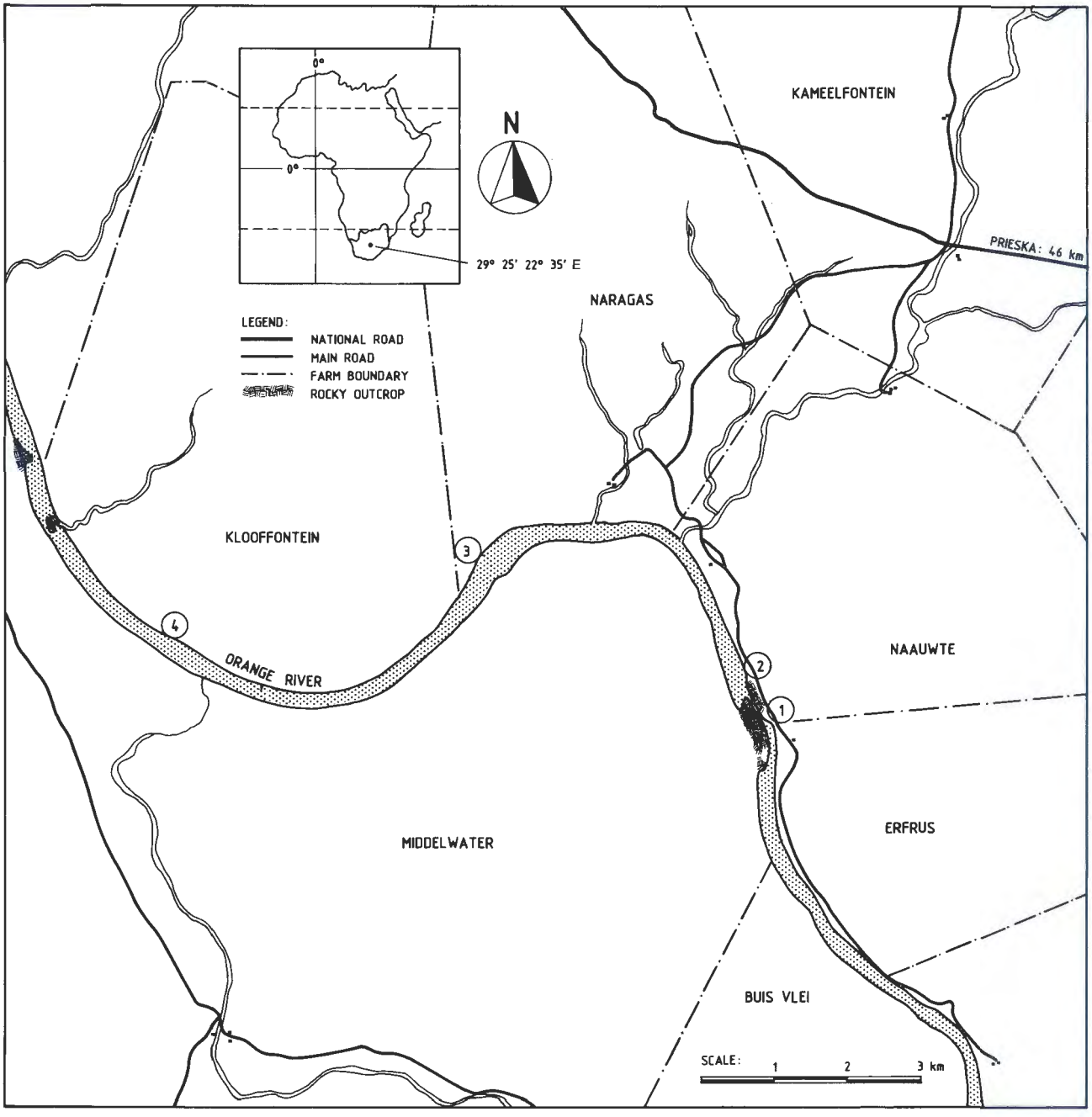

FIG. 1 The Orange River showing sampling stations,
(1) 200 m downstream from application point of *B.t.i.*;
(2) Farm Naauwte;
(3) Farm Naragas;
(4) Farm Klooffontein

the S.W. extreme of the Kaapvaal craton and the river passes through rocks of the lower Transvaal supergroup dominated by shelf carbonates with minor shales and siltstones (Vajner, 1974). The river cuts through rocky gorges formed in Ventersdorp lava and forms rapids interspersed with long stretches of swiftly flowing but unbroken water. As 95 % of the precipitation of the river catchment (excluding the Vaal River) occurs upstream of Lake le Roux (Pitman, Middleton & Midgley, 1981), the flow of the lower Orange River is almost entirely dependent on water released from this lake. The sampling sites chosen were far enough downstream from the lake to negate the effects of water flow regulation applied periodically for the control of Simuliidae further upstream (Howell *et al.*, 1981).

Throughout our survey the flow of the Orange River was 38 m^3/s. The water was mildly turbid (24–26 NTU) with a pH of 7,5 and a conductivity of 22 mS/m. Temperatures in the flowing water ranged from 19,8 °C at 15h00 on the 18th to 16,8 °C at 07h40 on the 20th April 1983.

An 11 km stretch of the Orange River between the boundary of the farm Erfrus and rapids on the farm Klooffontein was selected as the treatment area (Fig. 1). The top end of a rocky gorge 1,4 km long and about 50 m wide at its narrowest point was chosen as the *B.t.i.* application site. Sampling station 1 was in the rocky gorge 200 m downstream from the application point. Sampling station 2 was at the lower end of the gorge 1,4

km from the application point. The river then widened to about 100 m with a sandy bed and there were scattered small rapids all along its course (Fig. 5). Station 3 was situated at the foot of a 190 m outcrop 6 km downstream from the application site, where large rapids extended the width of the river (*c.* 200 m) and were *c.* 300 m in length. Station 4 was a further 5 km downstream where the river was about 150 m wide with short intermittent rapids. Sampling and treatment of the river took place between 18 and 24 April 1983.

Application of B.t.i. *and sampling methods*

To ensure the required spore concentration of *B.t.i.* (1,6 ppm/10 min) 38 ℓ of the concentrate were diluted with 82 ℓ of river water. The diluted *B.t.i.* was dispersed into the mainstream of the river, 200 m upstream of Station 1 between 09h41 and 09h51 on 21 April 1983 (Fig. 2 & 3). To determine the rate of surface water flow, styrofoam chips were released into the river at the start of the *B.t.i.* application (Fig. 3 & 4).

Both before the application of *B.t.i.* and 24 hours afterwards the fauna from a pooled sample of 4 small stones-in-current was collected at Station 1. At Stations 2, 3 and 4 randomly-collected samples of 5 stones-in-current were taken before the application of *B.t.i.* and at various time intervals afterwards (Tables 2–5). Fauna from each stone was separately scraped into a hand net of 92 μm mesh and all samples were fixed in 4 % formaldehyde. Estimates of stone surface area were made following Moor (1982a), and laboratory analysis of faunal material was carried out according to Car & Moor (1984).

Fauna drifting in the flowing water was collected in 92 μm mesh nets with 2 water-wheel drift samplers (Pearson & Kramer, 1969), as modified by Chutter (1975). The drift samplers were placed at the bottom end of the gorge at Station 2 in swiftly flowing water near the bank of the river and at the lower end of the rapids at Station 3 in fast flowing water 4 m from the river bank. Drift samples were collected before the application of *B.t.i.* and during the entire day after. The drifting animals collected were recorded as numbers/10 m^3 of water sampled (Table 6).

Drift samples were found to contain large quantities of blue-green algae (*Microcystis* sp.) which had to be separated from other organic matter. By centrifuging the samples at 300 rpm for 10 min it was found that the lighter floating *Microcystis* sp. could easily be siphoned off from the heavier invertebrates.

Statistical comparisons of faunal densities at times before and after the application of *B.t.i.* were made using the Mann-Whitney U-test (Elliott, 1977) and by comparing the median values of simuliid densities on stones.

Results and Discussion

Population estimates of benthic faunal densities before and after the application of B.t.i.

A decrease in simuliid larvae occurred 200 m below the point where *B.t.i.* was applied (Table 1). There was also an overall numerical increase in other invertebrate animals, suggesting that some colonization of the vacated stones-in-current habitat may have occurred.

At Station 2 on the farm Naauwte, where no samples were collected prior to the application of *B.t.i.*, numerous dead *S. chutteri* larvae were found on stones (Table 2).

In preserved samples, larvae which had been killed by *B.t.i.* showed no muscle tonus and were completely flaccid. They were therefore easily separated from larvae which were alive prior to collection and had retained their muscle tonus, giving them a turgid appearance. Fig. 6 & 7 show live and *B.t.i.*-killed larvae on substrates taken from the river and photographed immediately. There was also a decrease in the density of *S. chutteri* and *S. (Afrosimulium) gariepense* de Meillon larvae between 22 and 24 April at Station 2. An increase in small simuliid larvae between these dates indicated that recolonization of suitable substrates by drift, a phenomenon previously recorded by Moor (1982 b), had occurred. The presence of a few dead *S. chutteri* larvae on 24 April (Table 2) suggests that larvae killed by *B.t.i.* could remain attached to substrates for several days before being washed off by the water flow.

TABLE 1 Comparison of the pooled benthos from samples of 4 stones collected at a site 200 m below the point where *B.t.i.* was applied on 21 April 1983, before and 24 hours after the application. Invertebrates expressed as numbers/1 000 cm^2 of stone surface area

Invertebrates	Before treatment 18 April	24 h after treatment	Percentage change
Simuliidae			
Simulium chutteri larvae	950	38	− 96
S. chutteri pupae	40	18	− 55
S. (Afrosimulium) gariepense larvae	9	2	− 78
S. (A). gariepense pupae	3	4	+ 33
Small Simuliidae larvae	194	50	− 74
Chironomidae			
Orthocladinae larvae	4	6	+ 50
Ephemeroptera nymphs			
Baetis glaucus	4	0	− 100
Tricorythus sp.	4	96	+ 2 300
Small Ephemeroptera	0	58	+ ∞
Trichoptera larvae			
Amphipsyche scottae	27	28	+ 4
Cheumatopsyche thomasseti	0	3	+ ∞
Aethaloptera maxima	0	1	+ ∞

TABLE 2 The benthic fauna from stones in current (numbers/5000 cm^2) at Station 2 on the farm Naauwte, 1,4 km downstream of the application site, 25 and 75 hours after application of *B.t.i.* on 21 April 1983

Invertibrates	22 April 25 h after application	24 April 75 h after application	Percentage change
Simuliidae			
Simulium chutteri larvae	765(529)*	106(6)	− 86
S. chutteri pupae	151	1	− 99
S. (A.) gariepense larvae	36(9)	1	− 97 S−**
Small larvae	86	165	+ 92
Chironomidae larvae			
Orthocladinae	72	53	− 26
Tanytarsini	18	0	− 100
Small Chironomidae	81	66	− 19
Ephemeroptera nymphs			
Centroptilum excisum	3	0	− 100
Baetis glaucus	21	4	− 81
Centroptiloides bifasciata	0	3	+ ∞
Tricorythus sp.	451	174	− 61
Small Ephemeroptera	46	14	− 70
Trichoptera larvae			
Amphipsyche scottae	361	198	− 45
Cheumatopsyche thomasseti	103	120	+ 17
Aethaloptera maxima	13	25	+ 92
Ecnomus thomasseti	3	0	− 100
Small Hydropsychidae	63	77	+ 22
Platyhelminthes			
Planaria sp.	0	5	+ ∞

* larvae in parentheses signify dead larvae

** S− = significantly less. Only *S. (A.) gariepense* larvae were significantly less ($P<0,05$), Mann Whitney U test, after 75 h

FIG. 2 Gravity feeding the *B.t.i.* emulsion via hosepipe into the river

FIG. 3 Applying *B.t.i.* into the mainstream of the river

FIG. 4 The Orange River viewing downstream from the site where *B.t.i.* was applied. Styrofoam chips used as markers to measure flow rate are seen in the centre of the plate

FIG. 5 The Orange River at Station 3 on the farm Naragas showing rapids where samples were collected

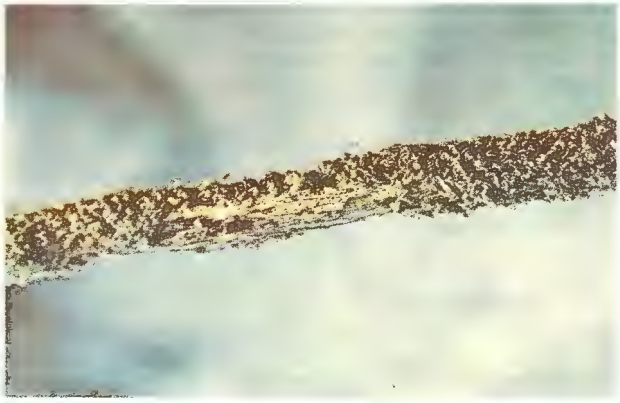

FIG. 6 Live *Simulium* spp. larvae on a piece of wood found wedged between some stones in the rapids

FIG. 7 Dead *Simulium* spp. larvae on a stone collected at Station 2, 25 h after the application of *B.t.i.*

TABLE 3 The benthic fauna from stones-in-current (numbers/5 000 cm²) at Station 3 on the farm Naragas and the percentage change and significance of change* (Mann-Whitney U-test) in faunal densities before and after treatment of the river with *B.t.i.*

Invertibrates	18 Apr Before treatment	22 Apr 29 h after treatment	24 Apr 73 h after treatment	Percentage change from 20–22 April	Percentage change from 22–24 April	Percentage change from 20–24 April
Simuliidae						
Simulium chutteri larvae	1 001	680(17)**	67	− 32	− 90	− 93
S. (A.) gariepense larvae	21	17	4	− 19	− 77	− 81
Small larvae	4 235	2 237(98)	1 018	− 47	− 55	− 76
Chironomidae larvae						
Orthocladinae	51	105	45	+ 106	− 57	− 12
Tanytarsini	123	237	70	+ 93	− 71	− 43
Ephemeroptera nymphs						
Centroptilum excisum	0	0	1	−	−	−
Baetis glaucus	55	33	48	− 40	+ 46	− 13
Centroptiloides bifasciata	5	11	0	+ 120	− 100	− 100
Tricorythus sp.	291	439	510	+ 51	+ 16	+ 75
Choroterpes sp.	29	0	8	− 100	+ 100	− 72
Small Ephemeroptera	59	144	67	+ 144	− 54	+ 14
Trichoptera larvae						
Amphipsyche scottae	148	494	178	+ 234	− 64	+ 20
Cheumatopsyche thomasseti	68	121	43	+ 78	− 65	− 37
Ecnomus thomasseti	68	20	34	− 71	+ 70	− 50
Orthotrichia sp.	31	66	11	+ 113	− 83	− 65
Ceraclea (Pseudoleptocerus) sp.	22	86	37	+ 291	− 57	+ 68
Small Hydropsychidae	162	335	165	+ 107	− 51	+ 2
Small Hydroptilidae	25	69	49	+ 176	− 29	+ 96

* Although numerically strikingly different, none of the faunal densities recorded at various times were significantly different (P<0,05)
** Dead larvae indicated in parenthesis

TABLE 4 The benthic fauna from stones-in-current (numbers/5 000 cm²) at Station 4 on the farm Klooffontein and the percentage change and significance of change* (Mann-Whitney U-test) in faunal densities before and after treatment of the river with *B.t.i.* on 21 April 1983

Invertebrates	18 Apr before treatment	22 Apr 32 h after treatment	24 Apr 80 h after treatment	Percentage change from 18–22 April	Percentage change from 22–24 April	Percentage change from 18–24 April
Simuliidae larvae						
Simulium chutteri	1 721	750	432	− 56	− 42	− 75 (S−)*
S. mcmahoni	6	0	0	− 100	− 100	− 100
S. (A.) gariepense	85	78	50	− 8	− 36	− 41
Small larvae	10 263	5 228	1 611	− 49	− 69	− 84 (S−)
Chironomidae larvae						
Orthocladinae	43	42	28	− 2	− 33	− 35
Tanytarsini	4	2	13	− 50	+ 550	+ 255
Ephemeroptera nymphs						
Baetis glaucus	70	42	12	− 40	− 71	− 83
Centroptiloides bifasciata	2	2	2	0	0	0
Tricorythus sp.	23	127	67	+ 452	− 47	+ 191
Choroterpes sp.	0	4	0	+ 100	− 100	0
Small Ephemeroptera	11	70	19	+ 536	− 73	+ 73
Trichoptera larvae						
Amphipsyche scottae	30	64	75	+ 113	+ 17	+ 150
Cheumatopsyche thomasseti	5	81	26	+ 1 520	− 68	+ 420
Aethaloptera maxima	0	6	8	+ 100	+ 33	+ 100
Ecnomus thomasseti	0	2	8	+ 100	+ 300	+ 100
Orthotrichia sp.	71	70	36	− 1	− 49	− 49
Ceraclea (Pseudoleptocerus) sp.	0	0	5	0	+ 100	+ 100
Small Hydropsychidae	3	82	33	+ 2 633	− 60	+ 1 000
Small Hydroptilidae	0	6	15	+ 100	+ 150	+ 100

* S− = Significantly less. Only *Simulium chutteri* larvae and small larvae were significantly less (P<0,05) at this station from the 18th to the 24th April

A decrease in Tanytarsini between 22 and 24 April at Station 2 indicated that this group of animals may show some susceptibility to *B.t.i.* toxins as previously noted by Car & Moor (1984). A decrease in most benthic invertebrates at Station 2 was discernible and only *Cheumatopsyche thomasseti* (Ulmer), *Aethaloptera maxima* Ulmer, small Hydropsychidae larvae and *Planaria* sp. showed an increase in numbers between 22 and 24 April (Table 2).

Six kilometres further downstream, on the farm Naragas, simuliid larvae killed by the *B.t.i.* formulation were also found 29 h after its application (Table 3). A decrease in all species of simuliid larvae occurred after the application of *B.t.i.* on 21 April. This decrease became more pronounced between 22 and 24 April. Tanytarsini did not show such a drastic numerical decrease at Station 3 as at Station 2, apparently because the concentration of *B.t.i.* was already too diluted to eradicate this group of animals here.

Although no dead simuliid larvae were recorded on the farm Klooffontein, a gradual decrease in simuliid larvae was discernible between 18 and 24 April (Table 4). The density of *Baetis glaucus* Agnew tended to decline but other invertebrates did not appear to be adversely affected by the *B.t.i.* application since there was a general increase in their numbers.

Very few of the numerical and percentage differences recorded in Tables 2–4 were significantly different ($P<0,05$) from each other. Only *S.(A.) gariepense* at Station 2 was significally less ($P<0,05$) between 25 and 75 h after the *B.t.i.* application, and at Station 4 only *S. chutteri* and small simuliid larvae were significantly lower ($P<0,05$) after the application of *B.t.i.* than they were before. This was apparently because the replicate samples of 5 stones-in-current collected at each station were not large enough to cover the occasional variations in faunal community distribution and indicate statistically significant differences. This would have been due to the fact that the river was wide at Station 3 and it was not possible to get into the main flowing channel to collect samples. The actual sites selected for stones-in-current habitats may have been in the marginal distributional range in sub-optimal simuliid habitats along this stretch of river. The Vaal River is smaller, with more homogeneous stones-in-current habitats, than the Orange River. Samples of 5 stones-in-current are sufficient to indicate significant statistical differences ($P=0,05$) in faunal densities in the Vaal River (Moor 1982b, Car & Moor 1984), but apparently were not large enough to show up statistically significant differences in the Orange River. This illustrates the importance of pilot studies to determine the spatial distribution of fauna within a particular river and the minimum required size of statistically comparable samples.

However, the presence of dead *Simulium* spp. larvae at several sampling stations confirmed the efficacy of the *B.t.i.* product, at the suggested concentration of 1,6 ppm/10 min and at a flow rate of 38 m^3/s, for at least 6 km downstream of the application site. The statistically significant decrease of *S. chutteri* and small simuliid larvae 11 km downstream from the application site indicates that the stones-in-current habitat selected at station 4 was more homogeneous and hence required a smaller sample size to show numerical faunal changes with time.

A general trend in the decrease of simuliid larvae after the application of *B.t.i.* was evident at all stations (Tables 2–4). A comparison of the median values of simuliid larval densities expressed as numbers/1000 cm^2 for each individual stone from each of the 5 stone samples is summarized in Table 5. It is clear from this that a trend of decreasing numbers of benthic simuliid larvae occurred at Stations 2, 3 and 4. The increase of small simuliid larvae at Station 2 could have been due to recruitment from drift as discussed above.

Faunal drift in the Orange River before and after treatment with B.t.i.

Faunal drift in the river, 1,4 km and 6 km below the application site of *B.t.i.* was of the same order of magnitude throughout the period surveyed (Table 6). At Station 2 there was a marginal increase in simuliid larvae around 1½–2½ h after the estimated time of arrival of the *B.t.i.*; an increase in the number of chironomid larvae and ephemeropteran nymphs in the drift approximately 2½ h after *B.t.i.* application was also notable. At Station 3 there was also a small increase of simuliid and chironomid drift around the estimated time of arrival of the *B.t.i.*

TABLE 5 Change in the median number of simuliid larvae on stones-in-current (extrapolated to express numbers/1 000 cm^2 of stone surface) at Stations 2, 3 and 4 between 18 and 24 April 1983. (—) indicates no samples were collected

Station and invertebrates	Before treatment 18 April	20 April	After treatment 22 April	24 April
Station 2 Naauwte				
Simulium chutteri larvae	—	—	148	16
S. (A.) gariepense larvae	—	—	5	0
Small larvae	—	—	6	13
Station 3 Naragas				
Simulium chutteri larvae	—	88	83	7
S. (A.) gariepense larvae	—	3	0	0
Small larvae	—	718	232	221
Station 4 Klooffontein				
Simulium chutteri larvae	271	—	204	92
S. (A.) gariepense larvae	14	—	9	10
Small larvae	1 530	—	1 080	323

The increase in faunal drift after the application of *B.t.i.* was not nearly as spectacular in the Orange River as in the Vaal River (Car & Moor, 1984). The generally much lower drift densities recorded in the present survey when compared with those of the Vaal River study (Car & Moor, 1984) could have resulted from the fact that drift samplers were placed in rapids rather than upstream or downstream of the actual run. A second factor which may have caused the lower drift densities was that the samplers were not placed in or near the main stream-flow but along the sides of the current near the bank of the river.

CONCLUSIONS AND RECOMMENDATIONS

Previous assessments of the effectiveness of *B.t.i.* on target and non-target organisms in South Africa revealed that in the Pienaars River high chloride concentrations (77 mg/ℓ) and high sewage effluent concentrations considerably reduced the toxicity of *B.t.i.* (Car, 1984). In the Vaal River, a low flow rate (6 m^3/s) decreased the downstream carry of *B.t.i.*, thus reducing its effectiveness against simuliid larvae (Car & Moor, 1984). Although chloride concentrations in the Vaal River were not measured in December 1982, water samples collected in both the Vaal and Orange Rivers in April 1983 revealed chloride concentrations of 76 and 21 mg/ℓ respectively for these 2 rivers. Thus high chloride concentrations in the Vaal River may also have limited the toxicity of *B.t.i.* in its immediate application area.

At a flow of 38 m^3/s and a chloride concentration of 21 mg/ℓ, *B.t.i.* was visibly effective in killing simuliid larvae 6 km downstream of its application site, and significant reductions ($P<0,05$) of *S. chutteri* and small larvae were recorded up to 11 km downstream. A decline in the efficacy of *B.t.i.* as one moves further downstream from the application site is well known (Molloy & Jamnback, 1981).

In South Africa formulations of *B.t.i.* have been tested against various non-target species as well as a number of *Simulium* species, including *S. adersi* Pomeroy, *S. chutteri, S. damnosum sensu lato* Theobald, *S.(A.) gariepense, S. hargreavesi* Gibbins and *S. mcmahoni* de Meillon (Car 1984, Car & Moor 1984, present study). Burges (1982) compiled a list of non-target fauna not susceptible to *B.t.i.* and concluded that it would be unnecessary to continue further testing of *B.t.i.* against non-target fauna. We disagree with this conclusion and recommend that, in each new area and situation where *B.t.i.* is to be applied for *Simulium* or mosquito control, it should be carefully screened against the co-existing aquatic fauna. This is because a disruption or eradication of certain species in a community could severely upset the ecologi-

TABLE 6 Drift of invertebrates (numbers/10 000 ℓ) in water samples collected in rapids at Station 2 and Station 3. *B.t.i.* applied at 09h41 on 21 April. Estimated time of arrival of *B.t.i.* indicated by *

Drift collected at Naauwte (Stn 2).																
Date	19 April					20 April					21 April					
Time sample collected			15h30	16h35	17h30						10h03	11h19*	12h39	13h40	15h06	
Duration (min)			79	55	74						88	69	64	49	72	
Volume sampled (ℓ)			2 038	1 242	1 811						1 797	1 650	1 538	1 283	2 234	
Simuliidae larvae			15	0	0						39	12	33	55	0	
Chironomidae larvae			15	8	11						22	6	0	31	0	
Ephemeroptera nymphs			10	8	22						17	6	7	23	0	
Trichoptera larvae			5	0	6						6	6	0	0	0	
Drift collected at Naragas (Stn 3)																
Date	19 April					20 April					21 April					
Time sample collected	13h06	14h58	15h54	16h52	17h49	18h48	08h41	10h54	13h08	15h05	11h38	12h48	14h04*	15h15	16h30	17h58
Duration (min)	110	105	50	50	50	50	61	110	126	110	63	59	68	61	65	81
Volume sampled (ℓ)	5 835	5 757	2 726	2 650	2 590	2 606	5 673	6 350	4 519	3 142	2 459	2 378	2 763	2 575	2 819	3 636
Simuliidae larvae	5	4	7	4	12	0	0	3	0	0	12	4	33	8	21	6
Chironomidae larvae	3	0	4	4	0	0	0	0	7	0	12	0	33	8	11	6
Ephemeroptera nymphs	12	4	22	4	19	35	6	5	11	0	0	4	0	0	0	0
Trichoptera larvae	3	0	7	0	0	0	0	0	2	0	0	4	0	0	0	0

cal balance and favour other pest species which were not a problem at that time. We would further suggest that the effects of *B.t.i.* on the rarer species, which are usually more sensitive to general pollution, should also be monitored. In the long term this will provide a better understanding of the functioning of particular river ecosystems and improve the management of rivers, especially as regards the prevention of outbreaks of pest and nuisance species. So far, all tests using *B.t.i.* against non-target organisms have been of short duration. As no studies on the intermediate and long term effects of *B.t.i.* on non-target fauna have been conducted to date, we urge that such studies should be initiated, e.g. the feeding of *B.t.i.*-killed larvae to aquatic predators and scavengers.

When comparing the ecological and financial costs of *B.t.i.* with those of other organophosphate and organochloride larvicidal formulations it should be remembered that the latter kill blackfly larvae and other non-target invertebrate fauna indiscriminately. Even more important is the fact that certain species in the *S. damnosum* complex have become immune to some of the organophosphate larvicides (Guillet, Escaffre, Ouedraogo & Quillévéré, 1980). It is, however, unlikely that simuliids will develop immunity to *B.t.i.* (Gaugler & Finney, 1982). The current cost of a *B.t.i.* application along a 30 km section of river would be *c.* R1 000, assuming a flow of 100 m^3/s and ensuring a concentration of 1,6 ppm/10 min, i.e. 96 litres of *B.t.i.* concentrate at R10-00/ℓ. Over the 600 km stretch of the Orange River where *Simulium* control is required 2 000 ℓ of *B.t.i.* would be needed, at a cost of *c.* R20 000. This assumes that 1 application would be effective for a 30 km stretch of river and that there would be 20 application sites. The whole 600 km section of river would not have to be treated as *S. chutteri* occurs only on substrates in the fast-flowing regions. The application costs are not included in this estimation. The cheapest method would be to involve riparian farmers with the applications of *B.t.i.* under the supervision of the State Veterinarian, and a series of up to 5 applications at weekly intervals would be required. This would bring the entire cost of a *B.t.i. Simulium* control programme to *c.* R100 000. Alternatively the *B.t.i.* could be applied directly into the river or from the air with either a helicopter or a fixed-wing aircraft. Howell *et al.* (1981) estimated that, even at that time, the cost of a series of 5 aerial applications of an organophosphate would be R1,5 million.

Although *B.t.i.* is commercially available, it would be relatively easy to produce it from various protein-rich materials (Anonymous, 1982). As this product would be used for both blackfly and mosquito control, local production could reduce the cost considerably. The highly specific action of the delta-endotoxin of *B.t.i.* against certain nematoceran Diptera (Culicidae, Simuliidae, Chironomidae) was believed to have been due to the alkaline gut pH of these fly larvae releasing and activating the toxin from its parasporal crystal (Lacey, Mulla & Dulmage, 1978). The susceptibility of adult mosquitoes to the parasporal crystal (Klowden, Held & Bulla, 1983), proved that a high gut pH was not necessary to activate the endotoxin. Because of the differing toxicities of formulations of *Bacillus thuringiensis* against various invertebrates (Padua, Ohba & Aizawa, 1980), a high degree of accuracy in the cultivation of *B.t.i.* formulations is essential. If possible, an accurate bioassay on pure strains of a target species, e.g. *S. chutteri* in South Africa, should also be implemented. If this can be achieved new isolates of *B.t.i.*, with improved toxicity of bacterial spores, can then be screened. To obtain an even more toxic *B.t.i.* through improved delta-endotoxin

production, genetic engineering techniques have been suggested (Gaugler & Finney, 1982).

If *B.t.i.* is to be used regularly for *Simulium* control in South Africa, ways of improving its efficacy must be considered. Gaugler & Molloy (1980) found that feeding in *Simulium* larvae was inhibited by increasing the particle concentration of water in a simulated stream to 50 mg/ℓ after an application of *B.t.i.* This ensured a longer gut retention of *B.t.i.* and increased larval mortality by nearly 90 %. Molloy, Gaugler & Jamnback (1981) also found that smaller *Simulium* species and early larval instars were more susceptible to *B.t.i.*, and that higher water temperatures led to increased activity of *B.t.i.*, resulting in higher mortality rates in larvae.

For the control of *Simulium* with *B.t.i.* it would therefore be advantageous to:

(a) Keep particle concentration in the river low prior to control.
(b) Induce feeding inhibition after the application of the product by raising the particle concentration in the river to around 50 mg/ℓ.
(c) Ensure high flow rates to increase carry of the product.
(d) Attempt to keep chloride and organic effluent concentrations low.
(e) Apply the product at the time of the year when larval populations are high and adult populations low.
(f) Take into account the water temperature, ensuring that this is not below 10 °C.

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