# Optimisation of *Bacillus thuringiensis* var. *israelensis* (Vectobac®) applications for the blackfly control programme on the Orange River, South Africa

# NA Rivers-Moore<sup>1\*</sup>, S Bangay<sup>2</sup> and RW Palmer<sup>3</sup>

<sup>1</sup> Institute for Water Research, Rhodes University, PO Box 94, Grahamstown 6140, South Africa <sup>2</sup> Department of Computer Science, Rhodes University, PO Box 94, Grahamstown 6140, South Africa <sup>3</sup> Nepid Consultants, PO Box 4349, White River 1240, South Africa

#### **Abstract**

The Orange River, South Africa's largest river, is a critical water resource for the country. In spite of the clear economic benefits of regulating river flows through a series of impoundments, one of the significant undesirable ecological consequences of this regulation has been the regular outbreaks of the pest blackfly species Simulium chutteri and S. damnosum s.l. (Diptera: Simuliidae). The current control programme, carried out by the South African National Department of Agriculture, uses regular applications, by helicopter, of the target-specific bacterial larvicide Bacillus thuringiensis var. israelensis. While cost-benefit analyses show significant benefits to the control programme, benefits could potentially be further increased through applying smaller volumes of larvicide in an optimised manner, which incorporates upstream residual amounts of pesticide through downstream carry. Using an optimisation technique applied in the West African Onchocerciasis Control Programme, to a 136 km stretch of the Orange River which includes 31 blackfly breeding sites, we demonstrate that 28.5% less larvicide could be used to potentially achieve the same control of blackfly. This translates into potential annual savings of between R540 000 and R1 800 000. A comparison of larvicide volumes estimated using traditional vs. optimised approaches at different discharges, illustrates that the savings on optimisation decline linearly with increasing flow volumes. Larvicide applications at the lowest discharge considered (40 m3·s<sup>-1</sup>) showed the greatest benefits from optimisations, with benefits remaining but decreasing to a theoretical 30% up to median flows of 100 m<sup>3</sup>·s<sup>-1</sup>. Given that almost 70% of flows in July are less than 100 m<sup>3</sup>·s<sup>-1</sup>, we suggest that an optimised approach is appropriate for the Orange River Blackfly Control Programme, particularly for flow volumes of less than 100 m<sup>3</sup>·s<sup>-1</sup>. We recommend that trials be undertaken over two reaches of the Orange River, one using the traditional approach, and another using the optimised approach, to test the efficacy of using optimised volumes of B.t.i.

**Keywords**: Simulium chutteri, Simulium damnosum, Orange River, flow regulation, Bacillus thuringiensis var. israelensis, optimisation

# Introduction

The Orange River is South Africa's largest river and surface water resource, with an average natural mean annual runoff (MAR) of 12 000 x 106 m³, a catchment area of about 1 000 000 km², and a longitudinal axis in excess of 1 000 km (Midgley et al., 1994). The need for assurance levels of flow in the agricultural, mining, industrial and municipal sectors has resulted in its naturally variable flows being highly regulated by a series of three major impoundments, and numerous weirs (Palmer, 1998). Orange River water is also transferred to the Great Fish River via an inter-basin transfer scheme. In spite of the economic benefits of such water management, there have been undesirable ecological consequences caused by outbreaks of pest blackflies (O'Keeffe and de Moor, 1988; Snaddon and Davies, 1998).

The blackfly problem in the Orange River began to escalate from the 1970s following completion of the Gariep and Vanderkloof Dams in the mid-reaches (Palmer, 1998). The outbreaks have a major impact on sheep farming (stock losses) and the grape industry (annoyance to labour) along the middle and

\* To whom all correspondence should be addressed.

**2** +27 46-6222428; fax: +27 46 622-9427;

e-mail: nick@iwr.ru.ac.za

Received 19 April 2007; accepted in revised form 21 January 2008.

lower Orange River. Estimates of the annual cost of blackfly outbreaks to sheep farming alone range from R67 m. (Mullins, 2005) to R88 m. (Palmer, 1997).

In 1992 the SA Department of Agriculture initiated a blackfly control programme along the middle and lower Orange River. The control area extends over a total river distance of about 850 km. Blackfly larvae are restricted to fast-flowing water, and highest numbers are found in riffles and rapids. There are at least 148 blackfly breeding sites within the control area, which equates to an average of one site every 5.7 km. The main larvicide used is the bacterial larvicide Vectobac®, which is based on formulations of *Bacillus thuringiensis* var. *israelensis*. Applications of larvicides are typically undertaken by helicopter, but bridges and a boat are sometimes also used (Palmer, 1998).

The volume of larvicide that is applied at each site depends on a number of factors, such as flow volume, water temperature, conductivity, turbidity, the abundance of planktonic algae, the formulation of the product and its associated shelf-life, and the species of blackfly. Although dose-response relations have been determined for a range of key variables (for example, Wilson et al., 2005), there is no reliable model that is capable of integrating such a wide range of variables to determine an appropriate dosage. Dosage calculations for the Orange River Control Programme are therefore determined empirically prior to each operational application. The experimental treatment comprises

two applications at different dosages, undertaken the day before operational treatment. The results of the experimental treatment are used to determine the dosages to be applied the following day for operational treatment. This method has proved to be the most reliable method of determining effective dosages (Palmer, 1997; 1998).

Another important consideration in the control programme is the distance downstream that larvicides remain effective, as this determines the spatial interval at which larvicides are applied. The downstream carry of a larvicide depends on a range of variables, the most important of which is flow, and also affected by, inter alia, silt load, turbidity and algae. This means that the rapids that are treated during an operational application depend on how much water is in the river at the time. The relationship between flow and the downstream carry of larvicides used in the Orange River Blackfly Control Programme are presented in Palmer et al. (1996). These relationships are used to identify which rapids should be excluded from any given operational treatment, as rapids within the expected downstream carry distance are excluded from treatment.

For the operational treatment, the helicopter pilot is provided with a list of numbers corresponding to rapids, indicating breeding sites to be treated, and the volume of larvicide to be applied at each site. This method can be improved by modifying the dose to take into account the relationship between dose and carry, as was achieved in blackfly control operations in West Africa (Chalifour et al., 1990). For example, instead of treating two rapids separately because they are located further than the expected carry, the same result can be achieved by applying an increased dose at the first site only. Likewise, isolated rapids could be treated effectively by applying a lower dose than is typically recommended. The relation between dose and carry can be determined experimentally.

With the major cost of the Orange River Blackfly Control Programme being the larvicide (Palmer, 1998), the aim of this paper was to apply the optimisation routines of Chalifour et al. (1990) to determine potential reductions in larvicide volumes used in the control of pest blackflies in the Orange River. If applied correctly, the method would reduce the

cost of the blackfly control programme, both in terms of larvicide volumes needed, as well as labour and helicopter flying time.

# Study area

The blackfly control area covers an 850 km stretch of the Orange River, from upstream of Hopetown to downstream of Onseepkans (Fig. 1). It was impractical to focus on the entire 850 km of Orange River in this paper. The focus was chosen to extend over 136 km of the Orange River, from Buchuberg Weir (upstream) to Uizip (downstream) (Fig. 2).

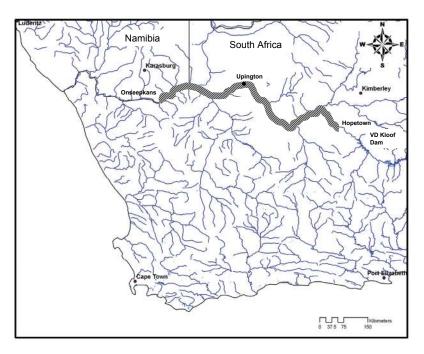


Figure 1
General locality map showing the full extent of the blackfly control programme in the middle and lower Orange River. The section used in this study is indicated by the buffered region on the Orange River.

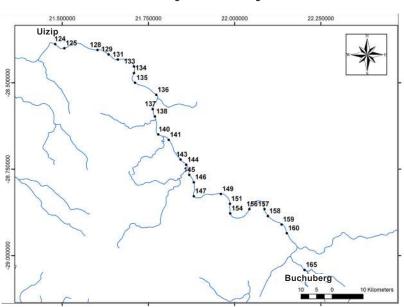


Figure 2
Section of the Orange River showing the position of the 31 breeding sites selected for this study

# Methods

#### **Data requirements**

The optimisation routines used by Chalifour et al. (1990) require a few basic inputs, viz. identification of blackfly breeding sites and downstream distances between different sites; flow volumes; downstream carry of larvicide; and the preferred  $LC_{50}$  concentration of larvicide.

A subset of the blackfly breeding sites was used, which had previously been identified by Palmer (1997) based on

TABLE 1
List of application sites with downstream distances, mean discharges during July, and volumes of
Vectobac <sup>®</sup> larvicide used in blackfly control programme

Site No	Site name	Latitude	Longitude	Distance from vd Kloof (km)	Distance from upstream rapid (km)	Carry (km)	Flow (m³·s-¹)
165	Buchuberg Weir	29°02'29.63"	22°12'10.70"	473.6		7.8	102
	Seekoeibaart	29°01'41.24"	22°11'15.21	475.8	2.2	7.8	102
	Luisdraai	29°00'46.00"	22°10'01.96"	478.6	2.8	7.8	102
	Dabep	28°58'02.23"	22°10.31.56"	484.6	6	7.8	102
160	Winstead 1	28°56'07.92"	22°09'05.44"	489.1	4.5	7.8	102
	Winstead 2	28°55'43.46"	22°09'00.40"	490.1	1	7.8	102
159	Buchuberg Town	28°54'36.99"	22°08'11.38"	492.6	2.5	7.8	102
158	Skerpioenpunt	28°53'09.84"	22°05'48.37"	499.8	7.2	7.8	102
157	Kheis	28°51'56.97"	22°05'12.26"	502.3	2.5	7.8	102
156	Rooisand	28°51'56.89"	22°02'34.68"	507.6	5.3	7.8	102
154	Groblershoop Bridge	28°52'47.04"	21°59'15.05"	514.8	7.2	7.8	102
151	Opwag	28°51'01.58"	21°59'11.35"	518.3	3.5	7.8	102
149	Rooilyf	28°49'19.34"	21°57'37.49"	523.3	5	7.8	102
147	Wegdraai	28°49'42.57"	21°52'54.47"	531.8	8.5	7.8	102
146	Sishen Railway Bridge	28°47'16.93"	21°52'54.30"	536.3	4.5	7.8	102
145	Saalkop	28°45'59.79"	21°52'07.36"	539.3	3	7.8	102
144	Perdelaagte	28°44'15.13"	21°51'34.92"	542.3	3	7.8	102
143	Volgraafsig	28°43'20.65"	21°50'35.63"	545.1	2.8	7.8	102
141	Kalkwerf	28°39'55.43"	21°48'31.54"	552.1	7	7.8	102
140	Gariep	28°38'59.05"	21°46'43.11"	556.6	4.5	7.8	102
138	Glimlag	28°35'51.35"	21°46'07.86"	562.1	5.5	7.8	102
137	Grootdrink	28°34'34.84"	21°45'45.84"	564.6	2.5	7.8	102
136	Pebble Beach	28°32'06.70"	21°46'22.63"	569.6	5	7.8	102
135	Lambrechtsdrift	28°30'00.86"	21°42'41.31"	576.8	7.2	7.8	102
134	Karos Weir	28°28'19.38"	21°42'29.76"	580.6	3.8	7.8	102
133	Albany	28°27'10.58"	21°42'29.23"	583.1	2.5	7.8	102
131	Swartkop	28°25'58.83"	21°39'41.31"	589.1	6	7.8	102
129	Vloer	28°25'04.80"	21°38'05.09"	592.3	3.2	7.8	102
128	Karos	28°24'19.00"	21°36'09.28"	595.9	3.6	7.8	102
125	Leerkrans	28°24'00.82"	21°30'22.82"	607.4	11.5	7.8	102
124	Uizip	28°23'17.89"	2128'47.98"	609.2	1.8	7.8	102

examinations of 1:50 000 map sheets, and verified using aerial surveys. From this, it was possible to calculate river channel distances between breeding sites. A flow volume of 102 m<sup>3</sup>·s<sup>-1</sup> was chosen for the 31 sites examined, which is the median daily flow volume for July, based on an analysis of the time series of mean daily flow volumes for the gauging weirs D7H005 (Upington Weir) and D7H008 (Buchuberg Weir) between 1978 (post-completion of impoundments on the Orange River) and 2005, which were obtained from the Department of Water Affairs and Forestry (DWAF, 2006). Since neither gauging weir completely describes flows within the study area, an average discharge was calculated using data from both weirs. Analyses of flow time series included basic descriptive statistics and flow duration curves. Dischargerelated downstream carry for each site was estimated based on the tables of downstream carry under a range of flow volumes determined by Palmer (1997). In this case, with an average flow volume of 102 m<sup>3</sup>·s<sup>-1</sup>, larvicide carry was estimated to be 7.8 km. Volumes of larvicide per treatment site were calculated based on mean flow volumes at each site, and using a recommended LC<sub>50</sub> concentration of 1.2 ppm (= 720 m $\ell$  per 1 m<sup>3</sup>·s<sup>-1</sup> of discharge) (Table 1).

#### Optimisation approach

Volumes of larvicide used within a traditional approach were calculated by looking at downstream carry distances in conjunction with distances between larvicides, and deciding which rapids would need applications. The LC  $_{50}$  concentration of 720 ml per 1 m³·s⁻¹of discharge was then used to calculate the volume of larvicide necessary at each of the rapids, based on flow volumes of 102 m³·s⁻¹. This approach would be similar to that currently used in many blackfly control programmes.

An optimised treatment strategy was calculated using the approach of Chalifour et al. (1990), based on experimental treatments carried out in the *Onchocerciasis* Control Programme in West Africa. This involved determining the contribution of downstream carry to  $LC_{50}$  concentrations of larvicide at each site, using a one-dimensional transport equation. This formed the inputs for calculating an optimal application path using graph theory, by determining a minimum cost path. In this case, cost was defined as the equivalent dosage to achieve the desired level of larval mortality. Ford's (1956) minimum cost path algorithm (Aho et al., 1983), as used by Chalifour et al. (1990), was applied to optimise larvicide applications on the 136 km focus

# TABLE 2 Descriptive statistics for mean daily flow rates at two gauging weirs on the Orange River for the month of July from 1978 to 2005

Statistic	D7H005	D7H008
Mean discharge (m <sup>3</sup> ·s <sup>-1</sup> )	106.95	97.90
Standard deviation (m <sup>3</sup> ·s <sup>-1</sup> )	88.91	90.56
Coefficient of variation (%)	83.13	92.50
Minimum discharge (m <sup>3</sup> ·s <sup>-1</sup> )	29.84	14.03
Maximum discharge (m <sup>3</sup> ·s <sup>-1</sup> )	552.83	517.65

TABLE 3 Flow durations (% time) for July at five discharges						
Discharge (m³·s-¹)	D7H005	D7H008				
40	85	68				
60	62	53				
80	46	42				
102	34	31				
120	28	27				

region of the Orange River. These algorithms were coded into a Java application. Initial code includes three arrays – rapids; downstream distances between rapids; and flow volumes (m³·s⁻¹) at each of the rapids – which are subsequently used in the optimisation routines. The optimisation routine was then compiled as a Java applet (http://delta.ru.ac.za/river/), and run within the Java development environment jdk1.5.0\_07 (Sun, 2006). At this stage, the optimisation can be applied to any river system, provided breeding sites, and distances between breeding sites, as well as flow volumes, are known. The arrays in the application can be readily edited with a text editor, to suit the particular river and application.

We applied the optimisation routines to the 31 breeding sites adjacent to each other at five different discharges (40, 60, 80, 102 and 120 m<sup>3</sup>·s<sup>-1</sup>). These were compared with typical larvicide volumes which would be applied using a traditional approach, in order to determine how the benefits of optimisation changed with discharge.

# Results

Basic descriptive statistics for both gauging weirs are provided in Table 2. The per cent time flows exceeding the 5 discharges considered in this study were also calculated using the flow duration curves (Fig. 3), and are shown in Table 3.

Using the traditional approach, a typical application of Vectobac® larvicide at a flow volume of  $102~\text{m}^3\cdot\text{s}^{-1}$ would require applications at 13 of the 31 breeding sites (42%), using a total of 955 ℓ of larvicide (Table 4). Assuming a cost of R100·ℓ⁻¹ of Vectobac®, each application would cost approximately R95 472, or R702·km⁻¹ of river. Using the optimised approach, a total of 11 of the 31 breeding sites (35%), and requiring 683 ℓ of larvicide, would be required (Table 4). This translates to a cost of R68 280 per application, or R502·km⁻¹.

When total volumes of larvicide needed using traditional and optimised applications at different discharges were com-

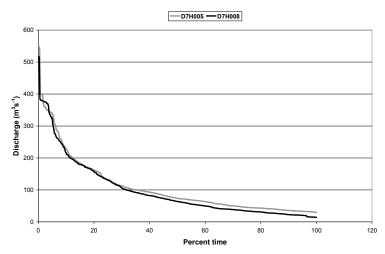


Figure 3

Flow duration curves for two gauging weirs on the Orange River within the study area showing the percentage time flows exceeding discharges of 0 m<sup>3</sup>·s·¹, based on mean daily flow data for the month of July from 1978-2005

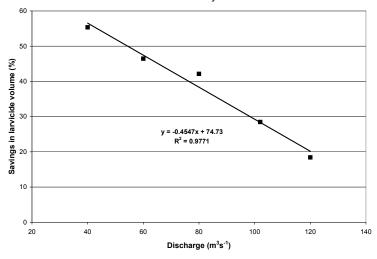


Figure 4

Percentage savings (traditional vs. optimised applications) made in larvicide volumes required to treat 31 blackfly breeding sites at different discharges in the Orange River, through aerial applications of Vectobac®

pared, a negative linear relationship best described the savings (Fig. 4; p < 0.01,  $R^2 = 0.98$ ). From this analysis, it is clear that savings (i.e. difference in volume between traditional vs. optimised approach as a percentage of total larvicide applied using traditional approach) decrease as discharge increases.

#### **Discussion**

The results of this study have shown that an optimised application approach can reduce the volumes of larvicide needed by between 20 and 55%, depending on flow. These results are comparable to those reported by Chalifour et al. (1990), whose optimisations for rivers in West Africa resulted in a reduction in the volume of larvicide needed by 48%, and a reduction in the number of sites to be treated by 32% (Chalifour et al., 1990). An implication of applying an optimised dosage approach is that aerial applications can be made during higher flows than at present, because for the equivalent volumes of larvicides currently used, higher flow volumes could be treated through lower

	Application volumes		ABLE 4 nal and opti	mised larvicio	de applicatio	ns
				treatment		treatment
Site No	Site Name	Distance from vd Kloof Dam (km)	Treatment	Volume of larvicide (ℓ)	Treatment	Volume of larvicide (ℓ)
165	Buchuberg Weir	473.6	1	73.44	1	76.585
	Seekoeibaart	475.8				
	Luisdraai	478.6				
	Dabep	484.6	1	73.44	1	74.683
160	Winstead 1	489.1				
	Winstead 2	490.1				
159	Buchuberg Town	492.6	1	73.44		
158	Skerpioenpunt	499.8			1	75.679
157	Kheis	502.3	1	73.44		
156	Rooisand	507.6		73.44		
154	Groblershoop Bridge	514.8	1		1	82.515
151	Opwag	518.3		73.44		
149	Rooilyf	523.3	1			
147	Wegdraai	531.8	1	73.44	1	51.699
146	Sishen Railway Bridge	536.3				
145	Saalkop	539.3		73.44	1	46.686
144	Perdelaagte	542.3	1			
143	Volgraafsig	545.1		73.44		
141	Kalkwerf	552.1	1		1	48.444
140	Gariep	556.6		73.44		
138	Glimlag	562.1	1		1	68.672
137	Grootdrink	564.6		73.44		
136	Pebble Beach	569.6		73.44		
135	Lambrechtsdrift	576.8	1	73.44	1	62.47
134	Karos Weir	580.6				
133	Albany	583.1		73.44		
131	Swartkop	589.1	1	73.44	1	63.763
129	Vloer	592.3				
128	Karos	595.9				
125	Leerkrans	607.4	1	73.44	1	31.644
124	Uizip	609.2				
Totals			13	954.72	11	682.84

volumes of larvicide per unit volume of river flow being required under an optimised approach, although the benefits and optimisation are greater at lower flows.

The Orange River Blackfly Control Programme focuses on 900 km of river, and if the optimisation were to be successfully applied, the direct savings of larvicide would be about 1 800  $\ell$  of larvicide per treatment. Assuming a nominal cost of larvicide of R100/ $\ell$ , the optimised approach would translate into direct savings of about R180 000 per treatment. Given that there are between 3 and 10 applications per year, the optimised approach could potentially reduce the costs of the control programme by between R540 000 and R1 800 000·a-1. These estimates do not include the reduced flying time, which would further increase benefits of the optimised approach. This would reduce the annual costs to the Orange River Blackfly Control Programme, and add to the already-proven economic benefits of this programme (Mullins, 2005).

Maximum benefits of using the optimised approach are reaped at lower flows. Downstream carry increases with increased flows, so that even in applying the traditional approach, it is possible to maximise the number of sites to be treated simply by 'eye-balling' them. In this case the number of sites to be treated using either of the approaches both stabilise at 11 sites at a discharge of 120 m³·s⁻¹. However, the optimum number of sites is reached far sooner using the optimised approach, and the discrepancy in volumes between traditional and optimised approaches increases as flow volumes decrease. Given that almost 70% of flows in July are less than 100 m³·s⁻¹, we suggest that an optimised approach is appropriate for the Orange River Blackfly Control Programme, particularly for flow volumes of less than 100 m³·s⁻¹. In this context, the potential savings outlined previously are likely to be conservative estimates.

Theoretically, volumes of larvicides used could be further optimised through the incorporation of temperature, conductivity and turbidity considerations in the LC<sub>50</sub> concentration calculations (Wilson et al., 2005). Additionally, increases in water temperatures and discharge have been shown to significantly enhance downstream carry of Vectobac® (Boisvert et al., 2001). However, we believe that this level of detail is unnecessary because in the Orange River Blackfly Control Programme these

variables are integrated when the test applications are undertaken the day before each operational application. The result from the test applications therefore provides the scaling factor for the recommended dosage. This is a practical approach that minimises the risks of application failure, and an adaptive way of calculating dosages. Note also that while we suggest the use of a consistent degree of accuracy, it must be borne in mind that these applications need to be adaptive, and absolute accuracy ultimately depends on the equipment used. Additionally, optimised applications require constant checking by the helicopter pilot, which may not be entirely practical given the difficult conditions and dangers (such as power lines) inherent in such low-level flying (Nel, 2006).

We recommend that the results of this optimisation be regarded as preliminary potential future savings. We also suggest that this be verified by dosing one section of river using a traditional approach, and another reach using the optimised volumes, and comparing mortalities using the 10-point scoring system of Palmer (1994). Such a study will show whether the conditions on the Orange River would be conducive to lower volumes of larvicides but applied using optimisations which consider downstream carry and residual effects of upstream applications. At this stage, there are also pointers to the value of maintaining and documenting pre- and post-treatment monitoring results of blackfly larval densities, as well as some justification for the collection of regular (daily) time series of at last water temperatures, but also possibly conductivities.

Should these trials be successful, the most pragmatic approach to optimising applications in the future would be to produce a booklet of lookup tables of recommended larvicide volumes for all the breeding sites for an expected range of discharges. Optimisation will be difficult in the highly anastomosed sections of the Orange River, particularly where these flow through dense reed beds, since under these conditions a high proportion of the larvicide is unlikely to reach downstream sites. In these cases, the traditional approach of application is more likely to succeed in controlling blackfly outbreaks, than the optimised approach.

# **Acknowledgements**

We thank the National Research Foundation of South Africa for financial assistance. The Water Research Commission, and in particular Steve Mitchell, are thanked for their support in this research. Messrs. Dirk Steenkamp, Kiewiet Viljoen and Johan Nell (Department of Agriculture, South Africa) are thanked for providing information on the blackfly control programme in the Orange River. Prof. Mike Burton (Department of Pure and Applied Mathematics, Rhodes University) is thanked for stimulating discussions. Prof. Alain Chalifour (Département de mathématiques et d'informatique, Université du Québec à Trois-Rivières, Quebec, Canada) is thanked for advice with the

optimisation model. The anonymous reviewers are thanked for their comments and inputs to this research.

#### References

- AHO AV, HOPCROFT JE and ULLMAN JD (1983) *Data Structures and Algorithms*. Addison-Wesley, Reading, Mass.
- BOISVERT M, BOISVERT J and AUBIN A (2001) Factors affecting black fly larval mortality and carry of two formulations of *Bacillus thuringiensis* subsp. *israelensis* tested in the same stream during a 3-year experiment. *Biocontrol Sci.Technol.* 11 711-725.
- CHALIFOUR A, BOISVERT J and BACK C (1990) Optimization of insecticide treatments in rivers: Application of graph theory for planning a black fly control program. Can. J. Fish. Aquat. Sci. 47 2049-2056.
- DWAF (2006) Department of Water Affairs and Forestry. Private Bag X313, Pretoria, 0001, South Africa.
- FORD LR JR (1956) *Network Flow Theory*. The Rand Corp. 293 pp.

  MIDGLEY DC, PITMAN WV and MIDDLETON BJ (1994) Surface
  Water Resources of South Africa 1990. Volume III: Orange-
- Water Resources of South Africa 1990. Volume III: Orange-Namaqualand. WRC Report No. 298/3.1/94. Water Research Commission, Pretoria, South Africa.
- MULLINS W (2007) Cost-benefit analysis. In: Palmer RW, N Rivers-Moore, W Mullins, V McPherson and L Hattingh (authors) Guidelines for Integrated Control of Pest Blackflies along the Orange River. WRC Report No. 1558/1/07. Water Research Commission, Pretoria, South Africa.
- NEL J (2006) Personal communication. Agriculture Control Programme, Department of Agriculture: Directorate of Resource Conservation, Pretoria, South Africa.
- O'KEEFFE JH and DE MOOR FC (1988) Changes in the physicochemistry and benthic invertebrates of the Great Fish River, South Africa, following an interbasin transfer of water. *Regulated Rivers:* Res. Manage. 2 39-55.
- PALMER RW (1994) A rapid method of estimating the abundance of immature blackflies (Diptera: Simuliidae). Onderstepoort J. Vet. Res. 61 117-126.
- PALMER RW (1997) Principles of Integrated Control of Blackflies (Diptera: Simuliidae) in South Africa. WRC Report No. 650/1/97. Water Research Commission, Pretoria, South Africa.
- PALMER RW (1998) An overview of black fly (Diptera: Simuliidae) control in the Orange River, South Africa. *Isr. J. Entomol.* **32** 99-110.
- PALMER RW, EDWARDES M and NEVILL EM (1996) Downstream carry of larvicides used in the control of pest blackflies (Diptera: Simuliidae) in the Orange River, South Africa. *J. Vector Ecol.* 21 (1) 37-47.
- SNADDON CD and DAVIES BR (1998) A preliminary assessment of the effects of a small South African inter-basin water transfer on discharge and invertebrate community structure. *Regulated Rivers: Res. Manage.* 14 421-441.
- SUN DEVELOPER NETWORK (SDN) (2006) <a href="http://java.sun.com">http://java.sun.com</a> WILSON MD, AK PABEY FJ, OSEI-ATWENEBOANA MY, BOAKYE
  - DA, OCRAN M, KURTAK DC, CHEKE RA, MENSAH GE, BIRKHOLD D and CIBULSKY R (2005) Field and laboratory studies on water conditions affecting the potency of Vectobac\* (Bacillus thuringiensis serotype H-14) against larvae of the blackfly, Simulium damnosum. Med. Vet. Entomol. 19 1-9.