The Impact of *Bacillus Thuringiensis Israelensis* (*Bti*) on Adult and Larvae Black Fly Populations

R. Mbewe[1], D. Pemba*[2], L. Kazembe[2], C. Mhango[2], S. Chiotha[2]

[1] University of Malawi, The Polytechnic, Physics and Biochemical sciences Department, P/Bag 303 Chichiri Blanytre 3, Malawi.

[2] University of Malawi, Chancellor College, Biology Department, P.O Box 280, Zomba, Malawi.

** pembadylo@cc.ac.mw*

Abstract

In the year 2007, the Ministry of Health (MoH) initiated a larviciding program using Bacillus thuringiensis israelensis (Bti) to mitigate the effects of black fly bites. This study was aimed at assessing the impact of Bti on adult and larvae black fly populations. Baseline data was collected prior to Bti application and after application Larva monitoring was done using four different substrates: nylon strips, rocks, and debri. Adult monitoring was carried out by human landing catches. Data analysis included descriptive summaries, t-tests, regression and Analysis of Variance (ANOVA). The analysis also included the assessment of the effect of Bti on adult flies and Larva density on substrates. All the statistical analysis were done at 5% significance level. The results showed statistically significant differences ($p < 0.001$) in populations of black fly before and after Bti application. Larva density was higher before Bti application and adult numbers were also high in that period. After Bti application a decrease in larva density was recorded and this associated with a gradual decrease in adult numbers. Bti had an impact on the larval population in that a decrease in larva population due to larviciding resulted in the decrease of adult population.

1 Introduction

Simulium spp. (Black fly) are distributed throughout the world in areas where conditions permit development of the larvae form. Many species are found in the subtropics and tropics where factors other than seasonal temperatures affect their development and abundance (Ward and Stanford, 1982). Black fly larvae are found in swiftly flowing, well-aerated water. Shallow mountain torrents are the favored breeding places. Some species breed in larger rivers but others live in temporary or semi-permanent streams (Palmer, 1997).

Black-flies have been a concern in many countries because they vector Onchocerciasis (River blindness). Onchocerciasis is an infection caused by the parasite *Onchocerca volvulus* (a nematode worm), spread by the bite of an infected black fly (Freeman and De Meillon, 1953). The symptoms of the disease include skin rash, eye lesions, squamous cell cancer and subcutaneous bumps under the skin. The most serious manifestation consists of lesions in the eye that can progress to blindness ((CDC), 2008; Enk, 2006). By 1994, an estimated eighteen to forty million people were affected worldwide, with about 270 000 having lost their sight (Courtright *et al*, 1994).

In Africa, the species responsible for transmitting the disease are various sibling species belonging to *Simulium damnosum* species complex and S. nevai (Kruger, *et al* 2004). Ninety-nine percent of the people living with Onchocerciasis are in Africa, and the disease is endemic in more than 25 nations across the central parts of Africa (CDC, 2008). *Simulium zombaense* and members of the S. *damnosum* complex occur naturally on Zomba plateau in Malawi, and all are potential vectors of O. *volvulus* (Pemba and Alezuyo, 2006).

Many Black fly control programs have been put in place to control the flies. These programs include larviciding to kill larvae and aerial spraying targeting adult flies. (World Bank, 2008; APOC, 2008). Mass Drug Administration (MDA) using Ivermectin has also been used for treatment and control of Onchocerciasis. Preventive measures of the disease include avoidance of infective bites of the black fly by using repellants such as Deet, and wearing long sleeve shirts and pants (CDC 2008). There is no vaccine against the disease.

Collecting data on *Simulium* flies before and after *Bti* application is an important aspect of monitoring to check the impact of the treatment on the targeted species. Although *Bti* is known to have worked in other places, it is important to monitor its impact under Malawian condition where environmental conditions and nature of the river in question is unique and no similar treatment has been done.

The main objective of the study therefore was to assessing the impact of *Bti* application on adult and larvae black fly populations.

2 Material and Methods

2.1 Study area

The study was done in Domasi part of Zomba District located in southern part of Malawi at 150 23' 0'' South and 350 20' 0'' East. Zomba has a land area of 2,580 km^2, comprising 3% of the total land area of Malawi. According to NSO (2008), the District has a total population of 591,903 representing about 5% of the national population. The population density is estimated at more than 209 persons per square kilometer but the density is higher in the urban area (NSO, 2008). Areas affected by black-flies are those around Zomba mountain-slopes, where the general topography varies from mountainous hilly regions to broad flat plains in the Upper Shire Valley and Lake Chilwa to the east.

Rivers and streams arising from springs in the upper portion of Zomba plateau are relatively short and swift, and flow over stones and boulders, within short distances before joining other tributaries and later Lake Chilwa. These features are characteristic breeding places for black-flies (Pemba and Alezuyo, 2006). The study focused on the Domasi River (about 20 kilometers) to the north of Zomba City which flows from the mountain and has varying water velocities down the water course. Domasi River was chosen because it is one of the rivers with the highest risk of Onchocerciasis vector sources warranting black fly control as was shown by the Rapid Epidemiology Assessment (REA) that was carried out in 2006 (Pemba and Alezuyo, 2006; WHO, 2006).

Sampling

Previous studies by Pemba and Alezuyo (2006) mapped out twelve breeding sites on Domasi River. The current study chose the middle course of the river on which seven breeding sites were identified. On each of the sampled breeding sites three samples were taken using purposive sampling. This was done to ensure that the monitoring covers the entire sampled stretch of the river having breeding sites taking into account the inherent environmental conditions that affect species density and composition. Water and air temperature, and flow rate of the river were recorded at sampling sites because they both affect larva occurrence and *Bti* performance (Figueiró *et al*., 2006). Adults were captured on a site close to where larva monitoring was done. This was done because adult black fly tends to concentrate close to their breeding sites (Bukaciski and Bukacinska, 2000).

Marked breeding sites for black-flies on Domasi River are shown in Figure 1.

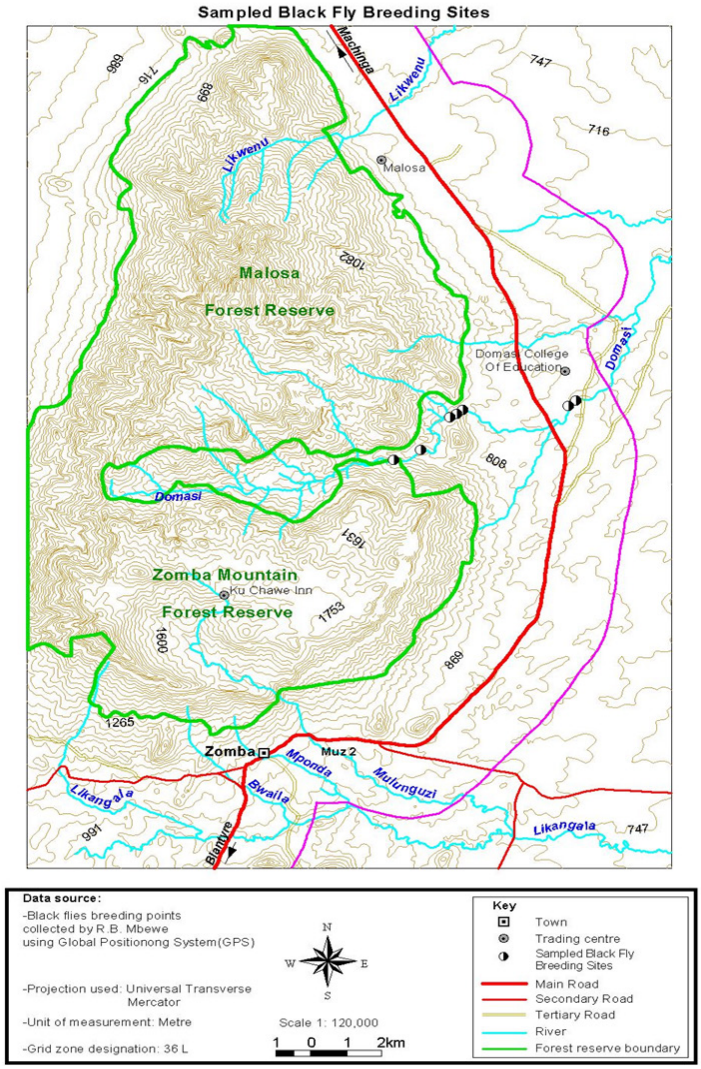

Figure 1: *Map showing black fly breeding sites on Domasi River on Zomba Mountain.*

2.2 Larvae monitoring

Data on black fly larvae were collected before *Bti* application in July 2008 to provide baseline data. Thereafter data were collected weekly for the entire study period of four months (August to November 2008).

Four substrates were used to monitor larva in this study: rocks, debris, strings and plastic boards. Choice of these substrates was partly determined by the nature of the river, duration of the study and their cost. Quantitative information on the population of black fly larva was obtained using substrates to capture larva. At every sampling point all 4 substrate types were deployed to sample larva. Initially two types of substrates were used, that is, plastic boards of 20cm × 20cm and nylon strips (threads). However Plastic boards were continually being stolen by fishermen fishing in the river every time they were set. As a result plastic board substrates were abandoned Plastic boards were replaced with rocks and debris (trailing vegetation) to capture larva in 2008. However, plastic boards were re-introduced in 2010

The colonized substrates were scored on a semi-logarithmically defined abundance scale (index classes) of 2-10 representing larva density according to the method of Palmer (1994). The number of larvae per 16cm2 were classified as indicated in Table 1.

Table 1: Scale of Larva density

Class	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Number of Larvae per 16cm^2	1 (1-2)	3 (3-4)	6 (5-9)	16 (10-22)	36 (23-58)	88 (59-120)	202 (121-310)	500 (311-800)	1050 (>800)

Data collected before and after *Bti* application was also used to determine changes and establish trends in the larva density. It was assumed that if *Bti* was effective at killing larva no further developmental stages would be observed. In this regard the adult population would decrease. However, if *Bti* did not have an effect on larvae, it would be expected that subsequent developmental stages would ensure, that is, pupae and then adult flies.

Another set of data on larva density in 2010 was used to compliment the 2008 data. This was done to further observe the impact of *Bti* rend in larva density after a long period of larviciding.

2.3 Adult monitoring

To determine adult black fly population, human bait was used to attract adult flies. This was done by exposing hands and legs to allow the flies to bite. Any fly landing on the exposed parts was captured using a pooter before it bites. The collection was done throughout the day with most catch between 6-9am and 4-6pm.

Data on adult black fly population was collected before *Bti* application to provide baseline data. After the first application of *Bti* data on adult fly population was continuously collected on weekly basis. To determine trend in adult population changes after larviciding data was collected on a site within a radius of 1km from the river. A radius of 1km was used because black-flies tend to concentrate close to the breeding sites (Hutchinson, 2008; Bukaciski and Bukacinska, 2000).

A total of three sites were used in the adult monitoring and the number of adult flies caught was recorded and the flies were kept as voucher specimen. The adult collecting sites were purposively chosen in relation to the breeding sites and were kept permanent throughout the study period. A total of 20 monitoring visits were made and 60 samples were collected.

2.4 *Bti* Application

To determine *Bti* dosage, water temperature was collected using a thermometer and flow rate which was calculated by multiplying the stream cross-sectional area (in square meters) by the flow velocity (in meters/second). From the temperature and calculated flow rate *Bti* dosage was determined be double dose of 0.72 - 1.44 liters per cumec and was applied for ten minutes. This contained 0.05-2.5 ppm of *Bti* (Technical use Bulletin for VectoBac 12AS Mosquito and black fly Larvicide). The application of *Bti* was done using Knapsack Sprayer and three applications of *Bti* were done in 2008 and four in 2010.

2.4 Data management and analysis

Data were entered into Microsoft Excel and SPSS version 12 for statistical analysis. Data analysis included descriptive summaries, t-tests, regression and Analysis of Variance (ANOVA). T-tests were done to compare mean densities on different site and substrates. Regression analysis was done to assess relationship between larva density and substrate type, site, year of monitoring and adult numbers. Factors like water temperature,

air temperature, and site were analyzed to assess how they affect black fly adult numbers and larva density. The analysis also included the assessment of the effect of *Bti* on larva density on substrates. All the statistical analysis was done at 5% significance level.

Data collected in the study area after larviciding was compared to the baseline data to determine the impact of *Bti* on the simuliids. Data collected after larviciding was analyzed to determine if there were any changes in population trend. 2010 data was compared to the 2008 data to see population changes in response to larviciding.

3 Results

Table 2 gives summaries of air and water temperatures collected during the study. The average air temperature before and after application was 20ºC and 26ºC, respectively. There was also an increase in water temperature from an average of 16ºC before application to 20ºC after the application. The increase in temperature was attributed to changes in seasons.

Before *Bti* application the mean larva density on the semi-logarithmically defined abundance scale (number per 16cm2) was 6.89 and dropped to 4.08 after application showing a significant decrease (P= <0.001). The adult flies captured had a mean of 19.6 (±6.8) before *Bti* application and dropped to 1.8 (±3.8) after application. This indicates a significant decrease in the mean adult numbers captured after *Bti* application (p<0.001).

Table 2: Summary of variables used in black fly population monitoring

Before *Bti* Application			**After *Bti* application**		
Variable	Sample (n)	Mean (± S.d)	Sample (n)	Mean (± S.d)	P- value
Air temperature ºC	45	20 ± 0	335	26 ± 3.4	< 0.001
Water temperature ºC	45	16 ± 0	335	20 ± 3.8	< 0.001
Laval density (#/16 cm^2)	43	6.89 ± 2.4	335	4.08 ± 2.90	< 0.001
Adult numbers	12	19.58 ± 6.8	49	1.84 ± 3.8	< 0.001

The summaries throughout the study show a decrease in the larva density. Larvae were abundant before *Bti* application in 2008 and significantly dropped after application Larva densities were at their lowest in 2010 as shown in Table 3 below.

Table 3: Summary of black fly Larva density (2008 and 2010)

Mean larva densities (#/16 cm^2)			
Period	*Before application*	*After application*	
Year	2008	2008	2010
Density	6.895 ± 2.3	4.087 ± 2.9	2.88 ± 0.76

3.1 Comparing Adults numbers and Larva density

The regression analysis comparing larva density and adult numbers showed that there was no significant relationship before application (p=0.238) and there was a significant relationship after *Bti* application

(p=0.005)

To determine the impact of *Bti* application on larva density and subsequently on adult population, regression analysis was performed. According to the p-values associated with the regression coefficients, it was observed that there was significant relationship between adult numbers captured and Larva density (p=0.005).

4 Discussion

Before *Bti* application, this study observed that both larvae and adult black fly populations were high. This observation suggested that as the larvae developed into adult, the number of adults in the environment increased. At the same time, as the number of adults increase, more eggs are laid in the river, and in turn, more larvae produced. According to the p-values associated with the regression coefficients, it was observed that there was no significant relationship between adult numbers captured and Larva density before *Bti* application (p=0.238) (Table 5). This finding shows that the changes in adult numbers captured before *Bti* application could not be associated with changes in Larva density alone, predation and seasonality can also affect the numbers.

However, there was a difference between what was observed and what was obtained after data analysis. Although the analysis showed no statistically significant relationship between adult numbers captured and Larva density before *Bti* application, the observation a marked relationship between adult and larvae in the environment. Since larvae depend on the adult fly to lay eggs that develop in to larvae and eventually into adults again, the application that affects the larvae breaks the life cycle and hence what happens to the larvae eventually is reflected at all levels of the cycle. Palmer (1997) observed that larvae have a life span of about three to six days on average while adult longevity in the field is estimated at eight to twelve days. This means that upon Bti application in the river it is expected to have the adult numbers dropping after a lag period of over 2 weeks from the day of application.

The observed a relationship between larvae and adult before Bti application could be due to the fact that the continuous life cycle of the flies without disturbance could not reflect the relationship significantly. Also the same observation could have been due to the fact that adult numbers captured were a small representation of the whole adult population. Of the entire adult population it was only the female human biting flies that were captured (Kettle, 1990) and this could be responsible for the observed lack of significant relationship. However, it was observed that an increase in the larva density resulted in an increase adult numbers captured.

On the other hand after *Bti* application there was a statistically significant relationship between larva density and adult numbers captured (p=0.005) (Table 6). This observation was due to the fact that as the larvae die (both male and female) in the river due to *Bti* application, the life cycle was disrupted. As there was no continuation of the life cycle the number of the adults in the environment began to drop. The general decrease of the adults in the environment involves the female biting fly as well, hence the observed decrease in the adults captured. These observations show that the larviciding suppressed the adult population.

5 Conclusions

The population changed over time and showed a decrease in the larva densities from July to November 2008. Similar results were obtained in larviciding done in 2010 from February to May where larva densities were low.

The study found that *Bti* had an impact on the black fly larvae populations in the river and consequently on the adult population in the environment. Hence population of Black fly decreased after *Bti* application at double dose of 0.72- 1.44 liters per cumec.

References

African Program for Onchocerciasis Control (APOC). (2008). Black fly available on: http://www.apoc.bf/en/index.htm.

Bukaciski, D. and Bukacinska, M. (2000). The impact of mass outbreaks of Black-flies (simuliidae) on the parental behaviour and breeding output of colonial common gulls (larrus Canus). *Annales Zoologici Fennici* 37: 43-49.

Centre for Disease Control (CDC). (2008). Factsheet, available on: www.cdc.gov/ncidod/dpd/parasites/onchocerciasis/factsht_onchocerciasis.pdf.

Centre for Disease Control (CDC). (2008). Information on International travel. Onchocerciasis (River blindness) available on: http://wwwn.cdc.gov/travel/yellowBookCh4-Onchocerciasis.aspx

Courtright, P., Johnston, K. and Chitsulo, L. (1994). A new focus of Onchocerciasis in Mwanza district. Blantyre, Malawi: Malawi Internation Centre for Eye Health, Barthstreet, London: ECIV9EL UK and International Eye Foundation.

Figueiró, R., Araújo-Coutinho, C.J., Gil azevedo1, L. H., Nascimento, E.S., Monteiro E.R.F. (2006). Spatial and temporal distribution of Black-flies (Diptera: Simuliidae) in the Itatiaia National Park, Brazil. *Neotrop. Entomol*. 35(4):542-550 http://www.scielo.br/scielo.php?

Freeman, P. and De Meillon, B. (1953). *Simulium* of the Ethiopian Region. London: The Trustee of the British Museum.

Kettle, D.S. (1990). *Medical and veterinary Entomology*. UK: Willingford.

Kruger, A., Kalinga, A.K., Post, R.J., at al. (2004). Two new cytoforms of the Simulium damnosum complex (Diptera: Simuliidae) from Malawi and Tanzania and potential Onchocerciasis vectors. Tropical Medicine & International Health. 9 (7): 805-811.

National Statistical Office (NSO). (2008). Housing and population census. Zomba: Government of Malawi.

Palmer, R. W. (1997). Principles of intergrated control of Black-flies (Diptera: Simulidae) in South Africa. WRC Report No. 650/1/97. Onderstepoort Veterinary ainstitute. South Africa.

Pemba, D. and Alezuyo, C. (2006). Zomba backfly outbreak report to the Ministry of Health, Lilongwe. Zomba: Biology Department, Chancellor College.

Ward, J.V., and Stanford, J.A. (1982). The Intermediate Disturbance Hypothesis: An Expansion for Biotic Diversity Patterns in Lotic Ecosystems: Dynamics of Lotic Ecosystems. Arbor Science Publishers. Ann Arbor 347-356.

Worldbank. (2008). Black-flies, Vector Services & Information available on: http://www.worldbank.org/afri/gper/partnerships.htm;

World Health Organisation (WHO). (2006). African Program for Onchocerciasis Control. Report of the twenty-six session of the Technical Consultative Committee (TCC). OuGaDougou, Bukina Faso.