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An experimental treatment of *Simulium posticatum* with *Bti* at selected sites on the River Stour, Dorset, 1998.

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Report date:	September 1998
Report to:	North Dorset District Council
IFE report ref:	RL/T04073C7/6
CEH project no:	T04073C7

Centre for Ecology & Hydrology
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1. In 1998, as in previous years, **TEKNAR HP-D (*Bti*) was found to be an effective simuliicide** when used against the larvae of *Simulium posticatum* under the conditions prevailing in the River Stour.

2. The populations of "**non-posticatum**" simuliids had, as usual, mostly emerged as adults by the end of March.

3. *Bti* application was again scheduled for the beginning of April when the overwintering populations had emerged and last instar larvae of *S.posticatum* had been found (showing that further delay in treatment would be undesirable).

4. The full river survey identified a range of sites with populations of *S. posticatum* larvae, these were treated on 2 April 1998.

5. Statistical analysis of samples of larvae, taken before and after application of *Bti*, showed that **the mean mortality was 42.6% at Blandford and 93.5% at Longham.**

1. INTRODUCTION

In 1993, the Health and Safety Executive (HSE) gave permission to treat the River Stour with *Bti*, where necessary, along the whole length of the river. Previously, restrictions to the areas treated were imposed by the HSE but following the successful experimental treatments in 1989, 1991 and 1992 clearance to treat for an experimental period of 4 years was given. In 1993, 1994, 1995, 1996 and 1997 successful treatments were carried out and samples were taken from sites down to Longham. A further extension permitted treatment In 1997 and 1998. Previous trials had shown no adverse effects on any fauna in the river apart from the target species *Simulium posticatum* (The Blandford Fly).

Treatment sites are no longer limited to a maximum of eight and the restriction of no spraying within 7 Km of the intake of Bournemouth Water Co. at Longham has been lifted. Despite the lifting of this latter constraint no sites between Canford school and Longham, have been treated.

The conduct of the present treatment took into account the "Guidelines for Biological Monitoring" put forward by the Pesticides Registration Section, 28 February 1990.

2. RECONNAISSANCE SURVEYS

2.1 Introduction

These surveys are designed to monitor the status of over-wintering populations of simuliids and the increase in density of *S. posticatum* larvae in order to determine the best time for treatment of the pest species. This is normally after the pupation and emergence of the overwintering larvae of other species and after the hatching of all of the eggs of *S. posticatum* but before pupation of that species.

In order to identify the above phase in the life cycle of the Blandford Fly, it is necessary to recognise first and, particularly, last instar larvae. The presence of first instar larvae would indicate that individuals were still hatching and that recruitment was continuing. Treatment at this stage would not affect the entire population. The presence of last instar larvae, in contrast, would indicate that pupation and emergence were imminent. Treatment must then be applied as soon as possible.

In 1998 a basic survey of sites was carried out on March 20 in order to establish that the larvae of *Simulium posticatum* were distributed in the usual manner and were at an appropriate state of development.

2.2 Methods

The standard sites at Blandford **(NGR ST 886 062)** and Longham **(NGR SZ 065 973)** were again chosen for the pre- and post-treatment samples, as they are known to have held large numbers of larvae in previous years and are near two of the main residential areas affected by the fly. In addition the site information is now building into a long term data base of treatment effects.

On the the 1 and 3 April quasi-quantitative samples of weed were taken, as usual, from the standard treatment and control sites at Blandford and Longham. In the laboratory, the simuliid larvae were identified and the numbers of *S. posticatum* larvae were recorded separately from the numbers of other simuliid larvae. First and last instars were noted. The wet weight of each sample of weed was recorded and the density of larvae was determined as numbers per gram of weed.

As a precaution on the 3rd of April the heavily infested control site at Blandford (carrier) was treated with 1l of *Bti*.

2.3 Results

2.3.1 Survey of River Stour for *S. posticatum* larvae 1998.

A sample of weed, taken by hand, was collected from fast flowing water at each site. The weed was thoroughly washed once in tap water and the water then poured through a 125 micrometre seive before examination under stereo microscope. Assessment of population density of larvae was as Abundant***, Moderate**, Small* or None. Approximate size/developmental state of larvae was judged by eye. Weed growth was adjudged to be lush, moderate or sparse and permission to treat was sought from the appropriate owners.

Blandford Control site - Sampled. good growth of weed in patch upstream of island. Very few 1-4 instar *S. posticatum* very few *S. erythrocephalum.* In consequence it was decided to use the carrier stream as the control site. And this site as the treatment site.

Blandford treatment site - No *Ranunculus* only some *Fontinalis* and algae. Many chironomid larvae on *Fontinalis* and few 1-3 instar *S. posticatum.*

Langton Long - Permission to treat OK but not treated because it is now thought that this site is covered by other treatments (Blandford and Charlton Marshall)..

Charlton Marshall - Moderate weed growth *S. posticatum.* Few last instar *S. erythrocephalum.* Larval population moderate, all instars. **

Clapcotts Farm - Weed growth moderate, Larval population moderate, larvae all instars, lots of last instar *S. erythrocephalum/S. lineatum.* Permission given to treat no bites reported in 1997 **.

Spetisbury - Weed growth moderate, Larval population moderate, larvae all instars. **

Shapwick - Weed growth sparse, Permission given to treat.

White Mill - Good weed growth upstream of bridge - No sample. No one contacted,.

Horse Field - Permission given to treat.

Corfe Mullen - Weed growth sparse.

Wimborne (Football ground) - Weed growth moderate larvae of all sizes **.

Julians Bridge - Weed growth lush. Larval population moderate, larvae all instars, chironomid larvae few. **

Canford School - Weed growth moderate, - Moderate weed growth, moderate numbers of 2-4 instar *S. posticatum.* Permission given to treat unless contacted. **

Longham control - Moderate weed growth upstream of bridge - No sample.

Longham Treatment - Little *Ranunculus* so sampled single clump of *Myriophyllum* and some submerged grass. Moderate numbers of 2-3 instar *S. posticatum* few large *S. erythrocephalum.***

Throop - Weed growth poor.

The general pattern appeared to be that larvae were a wider spread of ages than usual, possibly due to good growth conditions in February.

3. DISCHARGE AND VELOCITIES

Discharge values were required for calculation of *Bti* dilution factors.

3.1 Methods

The Wessex region of theEnvironment Agency are unable to provide discharge values at the prescribed sampling/application points as there are only two continuous gauging stations on the Stour, one at Hammoon a considerable distance upstream of Blandford and a second at Throop - potentially the furthest downstream site for treatment. The Agency were, however, extremely helpful having, in previous years, supplied maps and graphs which established that, with care, approximate interpolation between gauging stations is reasonable. With the experience gained and the fact that more sites need to be treated, interpolation of the Environment Agency gauging stations results is now used, as routine, to calculate quantities of *Bti* to be added at each site.

3.2 Results

The discharge of the River Stour at Hammoon and Throop, in March 1998, is given in Figure (1).

On treatment day (2 April 1996) the flow at Hammoon was 2.5 > m^3s^{-1} and at Throop 8.7 >m^3s^{-1} .

4. *Bti* APPLICATION

4.1 Methods and quantities

The above flows would require TEKNAR HP-D loadings of 1.2 l and 4.3 l respectively to achieve the desired concentration of **0.8ppm**. Blandford is well downstream of Hammoon and under the prevailing stable flow conditions of the time it was estimated that the discharge would exceed that at Hammoon by at least 50%. As in previous years treatment levels were conservative and ranged from a minimum of **1.8** l of *Bti* at Blandford to a maximum of **4** l of *Bti* at Throop.

The TEKNAR HP-D was carried to the sites as measured doses in closed containers and mixed in 20 l knapsack sprayers with sieved river water. The material was sprayed, by a qualified operative, who, when possible, traversed the river approximately ten times during the application period. The jet of the spraying equipment was totally submerged beneath the water surface to avoid spray drift or loss. At some points, where conditions rendered access to the river dangerous, mixing was achieved by introducing the required quantity of TEKNAR HP-D to a turbulent sluice or weir from a bridge or other vantage point. The sites were treated sequentially starting at Throop, the furthest downstream at 10.30 hr and ultimately treating , Blandford, the most upstream site on the river, at 16.00 hr on 2 April.

The quantities of TEKNAR HP-D required, in litres, to achieve concentrations of 0.8 ppm over ten minutes was calculated from the manufacturer's formula:

Volume (litres) = 0.48*Flow (cumec) and was as follows -

- Blandford main river	1.8 l
- Blandford carrier	1.0 l
- Charlton Marshall	2.0 l
- Clapcott's Farm	
Middle channel	1.5 l
West channel	0.5 l
- Spetisbury	2.5 l
- Shapwick	2.5 l
- Millmore Farm	2.5 l
- Whitemill farm	2.5 l
- Corfe Mullen	2.5 l
- Wimborne	3.5l
- Canford School	
Main river	4.0 l
Carrier	1.0 l
- Longham	4.0 l
- Muscliffe	4.0 l
- Throop	4.0 l

A total of **39.8 litres** of TEKNAR HP-D was added to the river on treatment day, **2 Apr 1998.** This is very similar to the amount in the previous year.

5. MONITORING THE EFFECTS OF *Bti* ON *SIMULIUM POSTICATUM*

5.1 Methods

30 weed samples were taken from each of the control and treatment sites at both Blandford and Longham on pre-treatment day (1 April 1998). At Blandford, the weed samples at the treatment site were taken 10 m below the proposed application point and at Longham this distance was 50 m. The sites chosen were dependent on site conditions such as presence of weed, suitability of conditions for larvae and safety of access. poor weed growth at the usual treatment site resulted in the control site at Blandford being shifted to the carrier stream.

Sampling was repeated on 3 Apr 1998, the day after treatment.

Samples were transported to the laboratory and the number of living larvae on each piece of weed was counted after identification into *S. posticatum* and other simuliid species. Weed samples were weighed after blotting dry. The method used was identical to that in previous years.

5.2 Results

5.2.1 Dead larvae

At Blandford, following treatment, dead *S. posticatum* larvae were recorded from the 30 weed samples at the treatment site on 3 Apr 1998. The number from the equivalent samples at Longham was dead larvae per 30 samples. No dead larvae were found in the Blandford control site samples but dead larvae were present in the Longham control site samples. As noted in 1993, 1994, 1995, 1996 & 1997 little interpretation of these results is possible as it is not known what (variable) proportion of dead larvae remain attached to the weed. It does show, however, that larvae were again effectively killed by *Bti* treatment.

The numbers of dead larvae on pre-treatment and control site samples are generally very small (much less than 1 per sample) although, as in 1996 and 1997, some (few) dead larvae were recorded from thirty control weed samples at Longham on post-treatment day (3 April 1996). Dead larvae examined had adopted the characteristic "stretched" appearance which we have come to associate with death following ingestion of *Bti*. It is thought that under the prevailing conditions of low flow/low turbidity/minimal weed growth/small larval population (reduced filtering capacity), the carry of the *Bti* was, as in 1995, 1996 and 1997, greater than previously encountered. In other words the larvae must have been killed by material carrying downstream from the Canford treatment point, a total distance of 7 km. In early trials substantial mortalities were recorded over carry distances of a little over 1km but in 1993 and 1994 there had been no evidence of mortality in the Longham control samples.

5.2.2 Density of living larvae

The densities at the four sites on pre-treatment day was, as in previous years, very patchy in distribution of larvae (Table 1). Control and treatment sites were within 50 m of each other at both locations (Blandford and Longham) and there was relatively little within location variation. There were however large differences between Blandford and Longham (Table 1).

The changes in density following treatment were tested statistically to see if they were significant. Initially the odds ratio method was applied and a t test used to compare means (Table 2).

5.2.3 Odds ratio method

This works on the premise that the ratio of the larval density before and after the treatment date should be the same at the control and treatment sites if there is no effect of the *Bti*, thus q, the 'odds ratio' coefficient, is determined as follows;

$$q = R_T/R_C = 1 \quad \text{where } R_T = x_{ta}/x_{tb} \text{ and } R_C = x_{ca}/x_{cb}$$

x_{ca} = mean density in the control site after treatment

x_{cb} = mean density in the control site before treatment

x_{ta} = mean density in the treatment site after treatment

x_{tb} = mean density in the treatment site before treatment

The data is log transformed as it is not normally distributed and the logarithm of x+1 is taken (where x is the density) owing to the presence of zero counts in some samples, giving $y = \log_{10}(x+1)$. The ratio now becomes the difference between before and after, D, (because we are dealing with logs), simply

$D_C = y_{ca} - y_{cb}$ for the control sites

and $D_T = y_{ta} - y_{tb}$ for the treatment sites.

If no treatment effect exists then, on average, $D_C = D_T$ or

$Q = D_T - D_C = 0$

Mathematically, $D_C = \log_{10} R_C$, $D_T = \log_{10} R_T$ and $Q = \log_{10} q$
so testing $Q = 0$ is equivalent to testing $q = 1$.

In practice the two tests are not the same since y_{cb} does not equal $\log_{10} x_{cb}$, etc.,because they are geometric means. However, the test of $Q = 0$ is preferable because it is effectively a test of differences rather than ratios, the latter being difficult to analyse.

$$Q = (y_{ta} - y_{tb}) - (y_{ca} - y_{cb})$$

and the standard error of Q is given by

$$SE(Q) = \sqrt{(SE_{TA}^2 + SE_{TB}^2 + SE_{CA}^2 + SE_{CB}^2)}$$

The test of $Q = 0$ is

$t = Q/SE(Q)$ with 116 degrees of freedom (n-1 for each of the four sites)

If densities have changed at the control site from before to after then the best estimate of the proportion of pre-treatment density left after application of *Bti* at the treatment site is

$$q = R_T/R_C$$

which is estimated by q_1 to q_2, where

$(q_1, q_2) = 10^{(Q \pm t SE(Q))} = \text{antilog}\,(Q \pm t\, SE(Q))$

The $\log_{10}$ x+1 values for mean density are given in Table 2.

The results were calculated for pre- and post- the retreatment as follows

Q = (0.49 -1.77)-(1.72-1.82)
= -1.821

The proportion of pre-treatment density remaining is
antilog Q = 0.065 or 6.5 %.

Thus the percentage kill at Longham was 93.5%.

Limits can be calculated from the formula, antilog (Q ± t SE(Q))
The SE(Q)= 0.1136 , therefore the limits are 0.915 and 0.949 (or 91.5%) and (94.9%).

The limits of the percentage kill are therefore 94.9% and 91.5%.

t = 677.82 with 116 df. p= <0.00001

This shows that the reduction in density at the Longham treatment site is significant p= <.00001

As the densities have changed at Blandford control, the best estimate of the proportion of pre-treatment density left after application of *Bti* at the treatment site is

Q = (0.176-0.564) - (1.204-1.351)
= -0.241

The proportion of pre-treatment density remaining is
antilog Q = 0.574

Thus the percentage kill at Blandford was 42.6%

Limits can be calculated from the formula, antilog (Q ± t SE(Q))
The SE(Q)= 0.276, therefore the limits are 1.083 and 0.700 (or 108%) and (70.0%).

The limits of the percentage kill are therefore 0 % and 70.0 %.

t = 2.08 with 116 df. p= 0.001

This shows that the reduction in density at the Blandford treatment site is significant p= >.001

6.2.4 Two sample t test

The t value tests for significance of the difference between two means. Samples are assumed to be independent and to come from normal distributions. As this is not the case the data requires log transformation. The calculations were performed twice, firstly assuming unequal within-time variability in log density and secondly assuming equal variances. At both Blandford and Longham there was no significant difference between pre- and post- treatment control site samples but, despite the small numbers of larvae at Blandford, there were highly significant decreases in density at both the treatment sites (Table 2).

7. DISCUSSION

Conditions for treatment of the river Stour in 1998 were ideal. Steady low flows made survey and treatment of the river easy. As usual a conservative approach to TEKNAR HP-D application was adopted. On 2 April 1998 several sites, including the monitoring sites at Blandford and Longham, were treated by the standard wading technique (see previous reports).

Significant reductions in mortality were observed at both Blandford and Longham treatment sites. At Longham there was a large reduction in densities following treatment.

The mean percentage kill achieved at **Blandford** was **43 %**. At **Longham** the kill was **93 %. These levels of mortality are similar to last year.**

Table 1 Densities of Blandford Fly larvae before and after treatment (numbers g^{-1})

	Blandford		Longham	
	Control	**Treatment**	**Control**	**Treatment**
01-Apr-98	32.1	3.7	72.2	63.5
03-Apr-98	21.6	0.80	55.0	3.8
%change	**32.7**	**78.3**	**23.8**	**94.0**

Table 2 Densities (Log 10+1), Student t values and significance levels by site *=significance level NS=not significant

	Pre-treatment	Post-treatment	t	p	Significance
Blandford Control	1.36	1.20	1.36	0.09	NS
Blandford Treatment	0.56	0.17	4.53	0.0001	***
Longham Control	1.82	1.70	1.67	0.04	*
Longham Treatment	1.77	0.47	14.48	0.0001	***

Acknowledgements

Our thanks go to R.T.Clarke for statistical advice and Dave Morgan of NDDC for assistance and support. Discharge data was kindly supplied by the South Western NRA (EA) (Wessex Region).

Fig.1

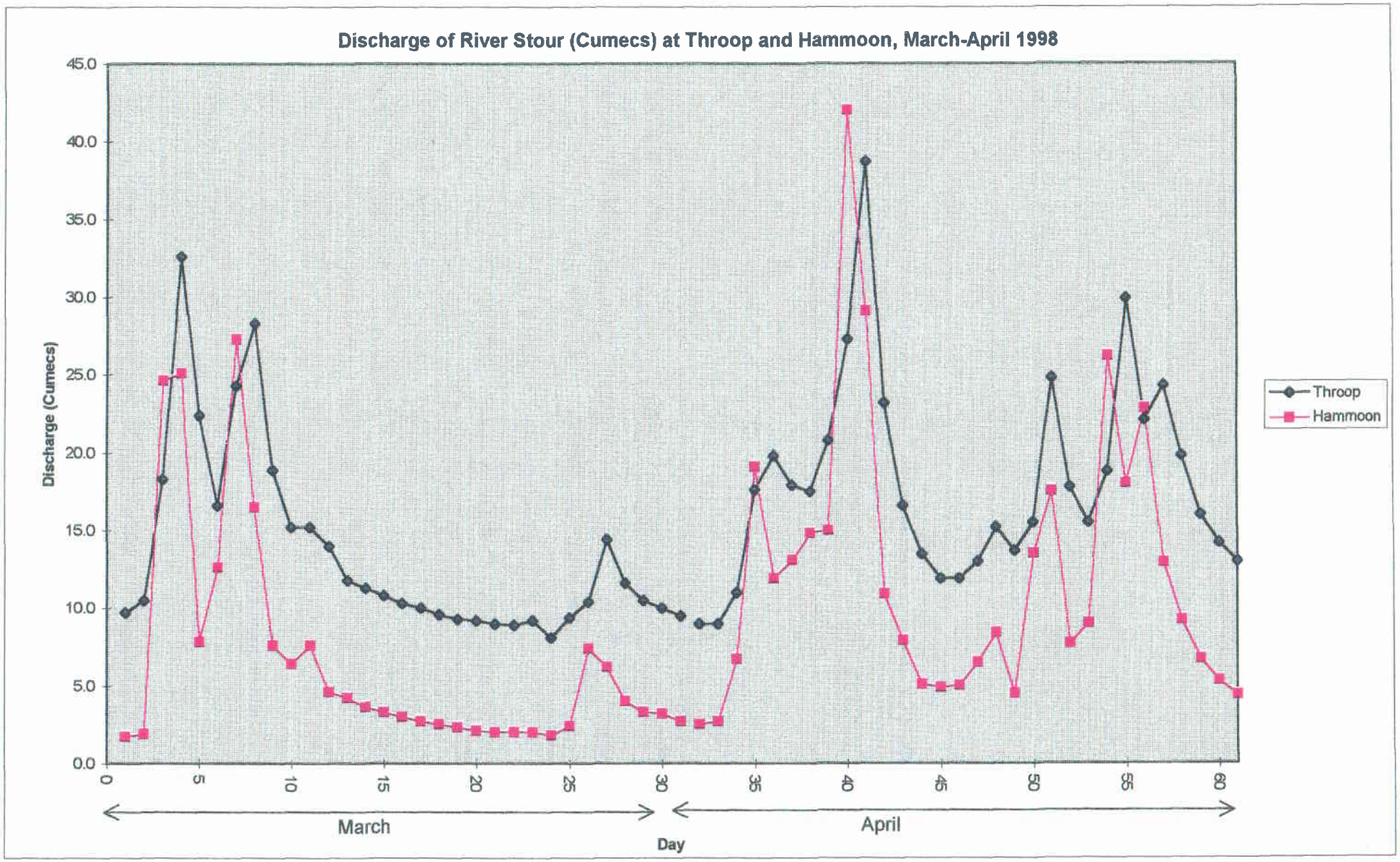
Discharge of River Stour (Cumecs) at Throop and Hammoon, March-April 1998
Discharge (Cumecs)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
5
10
15
20
25
30
35
40
45
50
55
60
March
April
Day
Throop
Hammoon

Fig. 2
Post-treatment changes in density of *S. posticatum* at Blandford and Longham 1998