Institute of Freshwater Ecology

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M Ladle PhD
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P Henville

Report to: North Dorset District Council
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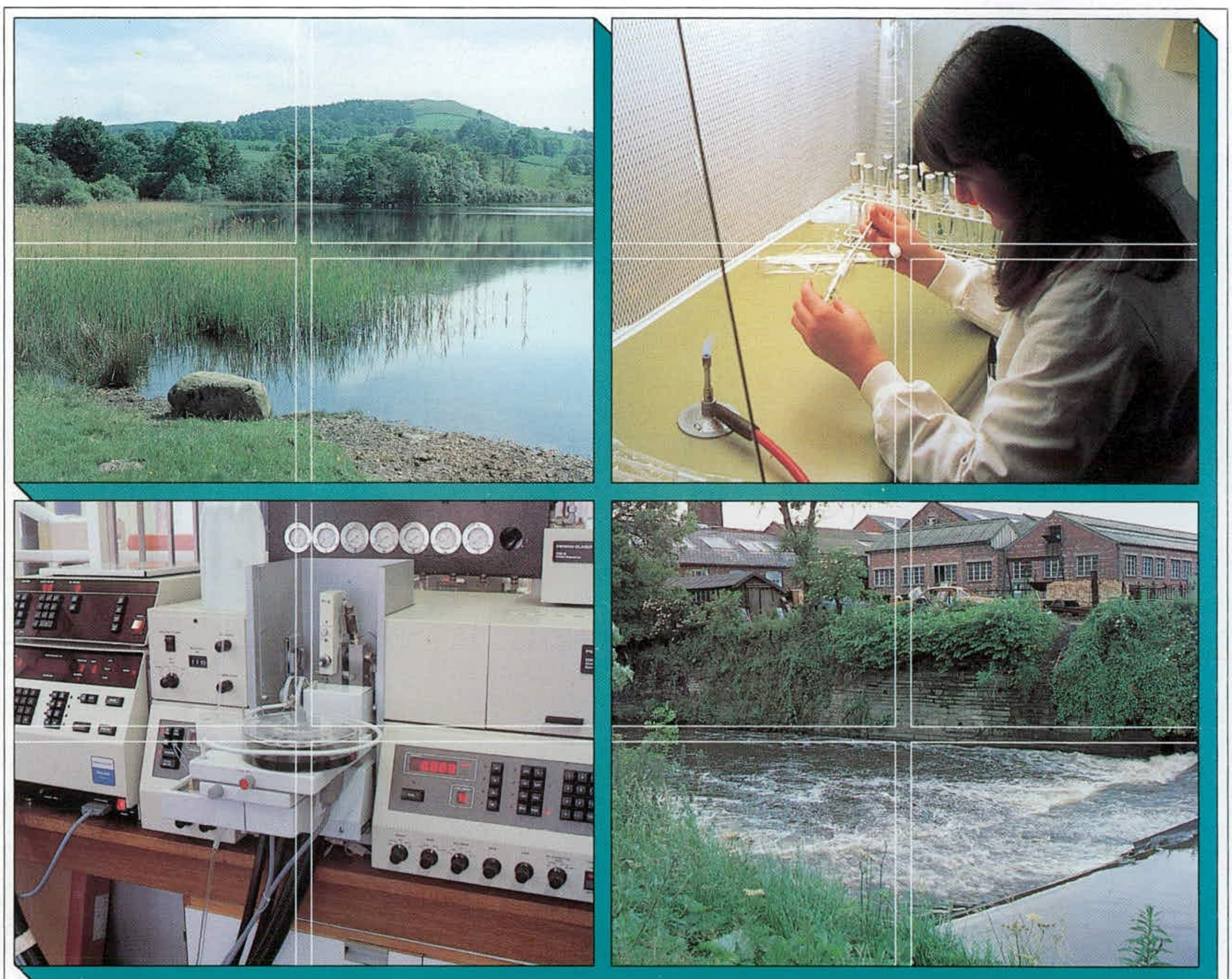

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Institute of Freshwater Ecology

River Laboratory
East Stoke, Wareham
Dorset BH20 6BB
United Kingdom

Telephone +44 (0)1929 462314
Facsimile +44 (0)1929 462180
Email

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Centre for Ecology & Hydrology
Institute of Freshwater Ecology
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Institute of Terrestrial Ecology
Institute of Virology & Environmental Microbiology

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River Laboratory, East Stoke, Wareham, Dorset BH20 6BB

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The Institute of Freshwater Ecology is part of the Centre For Ecology and Hydrology Directorate of the Natural Environment Research Council.

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 and 1996 successful treatments were carried out and samples were taken from sites down to Longham. A further extension permitted treatment 1n 1997. 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 1997 a basic survey of sites was carried out on March 17 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 7th of April the control sites at Blandford (carrier) and Longham were dosed with 1l and 2l of *Bti* respectively.

2.3 Results

2.3.1 Survey of River Stour for *S. posticatum* larvae 1997.

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 - Weed growth lush, larval population abundant, larvae 0.25 grown with some 1st instar. ***

Blandford treatment site - Weed growth sparse, larval population abundant, larvae 0.25 grown. ***

Langton Long - Permission to treat OK.

Charlton Marshall - Weed growth sparse, Larval population moderate, 0.25 grown. **

Clapcotts Farm - Weed growth sparse, Larval population moderate, larvae 0.25 grown. **. Permission given to treat no bites reported in 1996.

Spetisbury - Weed growth moderate, Larval population moderate, larvae 0.25 grown. **

Shapwick - Weed growth sparse, Permission given to treat but beware of young children on treatment day.

White Mill - Weed growth sparse. No one contacted,.

Horse Field - Permission given to treat.

Corfe Mullen - Weed growth sparse.

Wimborne (Football ground) - Weed growth poor.

Julians Bridge - Weed growth lush (below bridge) - sparse (above bridge), Larval population small, larvae 0.25 grown, chironomid larvae moderate. *

Canford School - Weed growth moderate, larval population small, larvae 0.25 grown, chironomid larvae abundant, moderate numbers of chironomid larvae. Permission given to treat unless contacted. *

Longham control - Weed growth lush.

Longham Treatment - Weed growth moderate, Larval population small, larvae 0.25 grown, chironomid larvae abundant. *

Throop - Weed growth poor.

The general pattern appeared to be that larvae were abundant at Blandford and the population diminished progressively with distance downstream.

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 1997, is given in Figure (1).

On treatment day (2 April 1996) the flow at Hammoon was 1.6 > 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 0.8 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 **1.2 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 11.00 hr and ultimately treating , Blandford, the most upstream site on the river, at 17.00 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	1.2 l
- Langton Long	1.2 l
- Charlton Marshall	2.0 l
- Clapcott's Farm	
Middle channel	1.5 l
West channel	0.5 l
- Spetisbury	2.5 l
- Millmore Farm	2.5l
- Corfe Mullen	2.5l
- Little Pamphill	3.5l
- Wimborne	3.5l
- Canford School	
Main river	4.0l
Carrier	1.0l
- Longham	4.0l
- Longham control	4.0l
- Muscliffe	nill
- Throop	4.5l

A total of 38.4 litres of TEKNAR HP-D was added to the river on treatment day, 2 Apr 1997. Due to the low flow conditions prevailing this is about half of that in the previous year (64 l in 1996).

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 1997). At Blandford, the weed samples at the treatment site were taken 50 m below the proposed application point and at Longham this distance was also 50 m. The sites chosen were dependent on site conditions such as presence of weed, suitability of conditions for larvae and safety of access.

Sampling was repeated on 3 Apr 1997, 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 1997. 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 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 killed by *Bti*.

Although the numbers of dead larvae on pre-treatment and control site samples are generally very small (much less than 1 per sample) again, as in 1996, there was an anomalous result with dead larvae 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 and 1996, much 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 sincey $_{cb}$ 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

$(q_1,q_2) = 10^{(Q \pm tSE(Q))}$ = antilog (Q ± 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

$Q = (0.99 - 1.81) - (1.54 - 1.75)$
$= -0.61$

The proportion of pre-treatment density remaining is
antilog Q = 0.24 or 24 %.

Thus the percentage kill at Longham was 76%.

Limits can be calculated from the formula, antilog $(Q \pm t\ SE(Q))$
The SE(Q)= 0.118 , therefore the limits are 0.856 and .577 (or 85.6%) and (57.7%).

The limits of the percentage kill are therefore 85.6% and 57.7%.

t = 5.16 with 116 df. p= 0.0001

This shows that the reduction in density at the Longham treatment site is significant p= >.0001

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

$Q = (1.66 - 2.07) - (0.97 - 1.02)$
$= -0.36$

The proportion of pre-treatment density remaining is
antilog Q = 0.44

Thus the percentage kill at Blandford was 56%

Limits can be calculated from the formula, antilog $(Q \pm t\ SE(Q))$
The SE(Q)= 0.101, therefore the limits are 0.695 and 0.305 (or 69.5.0%) and (30.5%).

The limits of the percentage kill are therefore 69.5 % and 30.5 %.

t = 3.56 with 116 df. p= 0.0001

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 Blandford, there was no significant difference between pre- and post- treatment control site samples but there was a significant decrease in density at the treatment site. At Longham, there were highly significant decreases in density at both the control and the treatment sites (Table 2).

7. DISCUSSION

Conditions for treatment of the river Stour in 1997 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 1997 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 but as in 1996 there was an unexpected high mortality at the control site. The condition of the observed dead larvae (characteristic form of rigor mortis) again confirmed that the control mortalities were undoubtedly due to the effects of TEKNAR HP-D.

The mean percentage kill achieved at **Blandford** was **56 %**. At **Longham** the kill was **76 %. These levels of mortality are similar to last year.**

The impression gained from local reports and from direct enquiries is that the cumulative effect of treatment, over the past five years has been very successful and once again far less people had been affected by the bites of the fly than in previous years. It is significant that 1997 was a year with excellent weather conditions in which biting activity would have been anticipated to be high, despite this few people were bitten.

CONCLUSION

1. In 1997, 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 end of March 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 3 April 1997.

5. Statistical analysis of samples of larvae, taken before and after application of *Bti*, showed that **the mean mortality was 67% at Blandford and 82% at Longham.**

Table 1 Densities of Blandford Fly larvae before and after treatment (numbers/g)

	Blandford		Longham	
	Control	Treatment	Control	Treatment
01-Apr-97	15.8	163.8	59.3	70.2
03-Apr-97	13.9	54.4	44.4	12.6
%change	**12.03**	**66.79**	**25.13**	**82.05**

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	1.02	0.97	0.39	0.5	NS
Blandford Treatment	2.07	1.66	4.78	0.0001	***
Longham Control	1.75	1.54	2.72	0.01	**
Longham Treatment	1.81	0.99	9.16	0.0001	***

Acknowledgements

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Figure 1. Discharge levels at River Stour gauging stations March/April 1997

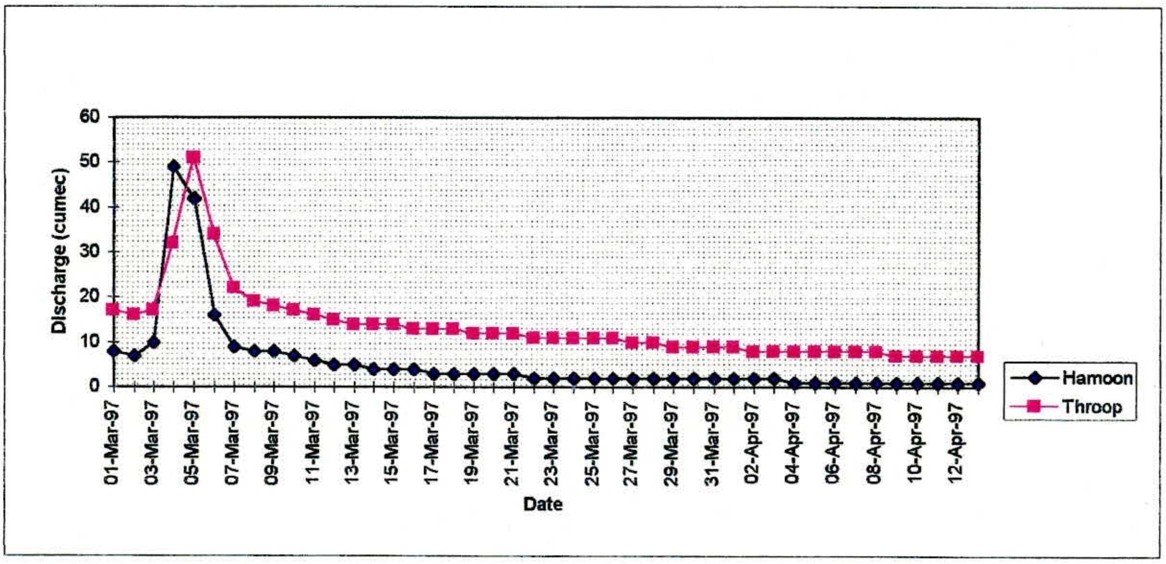
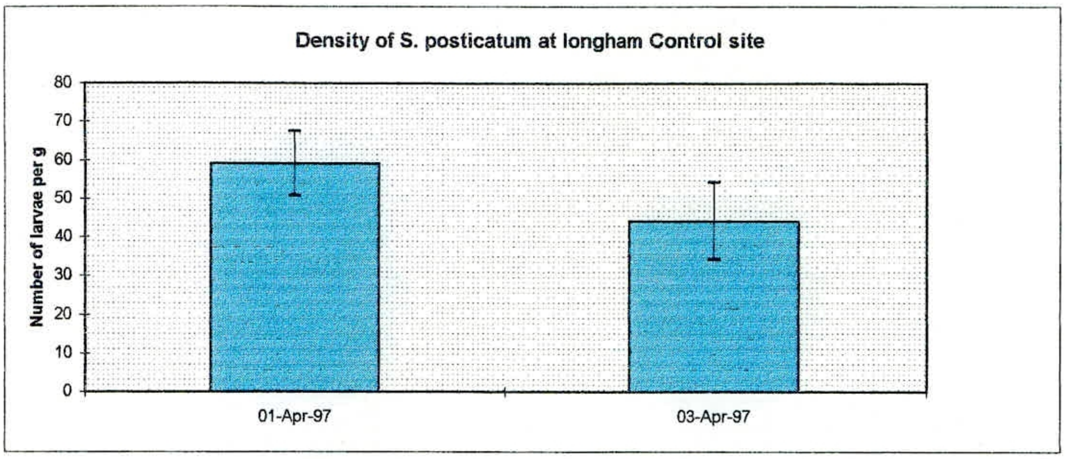
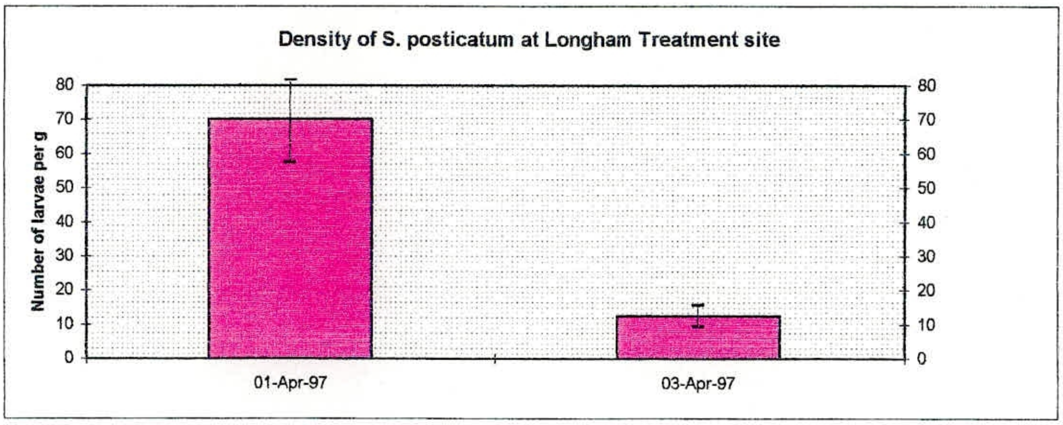
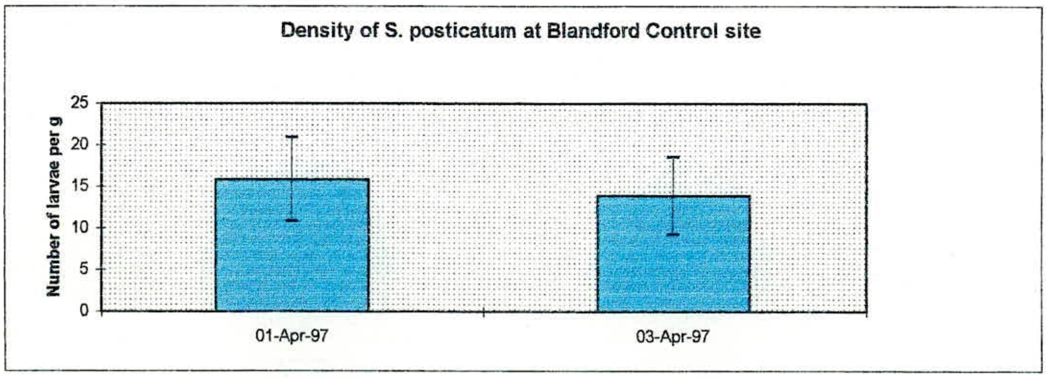
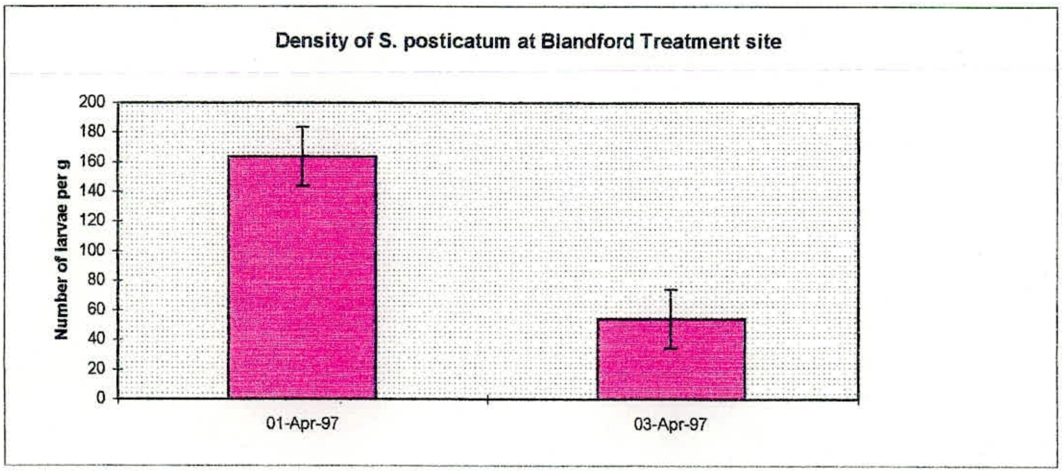


Figure 2. Histograms with 95% confidence limits showing densities of larvae before and after treatment









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