A FEASIBILITY STUDY OF THE CONTROL OF ADULTS OF HELIOTHIS SPP. BY DRIFTING A CLOUD OF MONOSIZED DROPLETS ACROSS A COTTON FIELD AT NIGHT

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma and, to the best of my knowledge and belief, contains no material previously published or written, or the result of work by another person, except where due reference is made.

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May, 1985

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SUMMARY

The possible control of adult Heliothis punctigera and Heliothis armigera in flight, by drifting a cloud of small droplets of insecticide across the canopy of a cotton crop at night, during the time of peak moth activity, has been investigated.

Two insecticides, fenitrothion technical and fenvalerate, were initially tested for their effectiveness against adult Heliothis spp. with a bioassay involving topical application. Fenvalerate proved to be the most effective, with an LD50 of 0.21 μg/g body weight, and was therefore chosen for use in the spray trials formulated as Sumicidin ULV (active ingredient: fenvalerate 40 g/l).

Six spray trials were carried out on consecutive nights, between 2100 and 2400 h. The trials were conducted under conditions of inversion and light winds - typical of the location (northwestern N.S.W.) and time of year (January to March). The droplet cloud was created by a spinning disc atomizer mounted on the back of a four-wheel-drive vehicle.

The moth population was estimated by flushing them from plants along transects within the crop and by pheromone traps: egg counts were also made. Analysis of the spray trials showed that it was possible to drift an insecticide at lethal concentrations across the crop canopy under inversion and light winds at night. Moth numbers were unusually low, so the effect of spraying on the moth population and egg lay could not be determined.

INTRODUCTION

The larvae of many species of Lepidopterous insects damage crops. The pest infestations are initiated either by immigrations of adults from other crops and/or native vegetation within the area, or over long distances (Wardaugh et al., 1980).

Two of the principal pests of the cotton industry in Australia are Heliothis armigera (Hubner) (cotton bollworm) and Heliothis punctigera (Wallengren) (cotton budworm) (Lepidoptera:Noctuidae) and their control is one of the major production costs. The larvae cause damage by feeding on the terminal leaf buds, squares and bolls of the cotton plant (Wilson & Waite, 1982), resulting in either a loss of plant uniformity or yield loss. A single adult female H. armigera can lay up to 700 eggs a day, with each resulting larva capable of destroying 18-24 fruiting points in its lifetime (Rendell, 1980). Insecticidal control of the species is usually based on a residual spray deposit to kill the larvae (Rendell, 1980). However, the larval feeding habits often make effective control with insecticides difficult because the parts of the plant on which they feed protect them from contact with the chemical (Broadley, 1977). For the residual spray deposit to be most effective it should be evenly distributed over the leaves on each plant within the crop. Even spray droplet cover and penetration of a crop is difficult to achieve and is a problem which is constantly before the spraying industry and research workers (Symmons, pers. comm.).

H. armigera has become resistant to a number of insecticides such as DDT (Broadley, 1977) and synthetic pyrethroids (Anon., 1983) but H. punctigera has remained susceptible. Resistance is caused by a combination of genetic factors, biological and behavioural characteristics and the method of insecticide application (Georghiou and Taylor, 1977).

Little attention has been given to controlling the larval infestations by spraying the adults and preventing oviposition. Night spraying against flying insects has been used successfully for some time against stored product pests in confined areas (Himel, 1969); the air space is filled with an aerosol spray released at the precise time of pest activity.

Similarly, control of Heliothis might be achieved by drifting a cloud of small insecticide droplets across a cotton crop when the moths are flying. Insects in flight are efficient collectors of small droplets (MacCuaig, 1958, 1962 and Spillman, 1976) and so perhaps the moths could be killed by a smaller amount of insecticide than is applied conventionally to a crop to kill the larvae. The conventional method, relying on a residual deposit of insecticide, is inefficient and requires a relatively large amount of chemical to be applied to a crop so that all parts of the crop are covered by a lethal dose.

The control of H. armigera by killing the moths in a cloud of small droplets (volume mean diameter (vmd) 40-50 μm) has been attempted in Thailand (Rendell, 1980). The cloud was produced by a portable, rotary atomiser sprayer mounted at a fixed point in a cotton field. The sprayer

was attached to a wind vane to ensure the nozzle oriented downwind and timed to operate for 30 seconds in each hour, from 1830 - 0630 hours. There were two control plots, both treated once a week by conventional deposition spraying. One of the plots was sprayed throughout the experimental period to control Heliothis larvae; the other was left unsprayed during the early season and then sprayed principally to control sucking insects. A uniform distribution of droplets was aimed for by having a swath width of 3 rows and carrying the sprayer along the rows with the head 1 m above the crop.

The effect of spraying was determined by estimating egg and larval numbers before and after spraying. The results showed that oviposition had been encouraged in the night - sprayed plot. The deposition of spray was uneven in the night - sprayed plot and overall Heliothis control was most successful in the plot that was sprayed uniformly once a week against the larvae.

This investigation followed on from Rendell's work and aimed to test whether an insecticide, as a cloud of small droplets, could be drifted over a cotton crop canopy at night so that it formed a uniform blanket above the crop.

The investigation also aimed to investigate whether the insecticide cloud produced in the manner described would kill H. punctigera and H. armigera moths.

The spraying technique, used in this investigation, would not be practical for the farmer, who is unlikely to be prepared to

circumnavigate crops for long periods at night. It is an initial investigation from which many questions arise on the biology and behaviour of Heliothis spp., the effects of sublethal doses of insecticide on these insects, as well as the economics and mechanics of a spray technique to be designed with the knowledge gained from this and subsequent investigations.

AIMS

This study aims to examine a number of hypotheses:

1. That a spray cloud consisting of small monosized droplets will remain aloft at a given height and across a specified area of cotton.

2. That the chemical remaining aloft will be sufficient to kill Heliothis adults across the entire plot.

3. That the adult population within the area sprayed will be suppressed.

4. That the egg lay will be reduced and hence the larval population be suppressed.

Chapter 1

BIOASSAY

1.1 INTRODUCTION

Little is known of the adulticidal qualities of many insecticides because most toxicity testing is carried out with larvae. Very few insecticides registered for use against Heliothis spp. in cotton are quoted as having a specific adult activity. To find a chemical that would kill adult Heliothis spp. for use in the spray trials, three insecticides were tested in bioassays involving topical application. They were fenitrothion, fenvalerate and cypermethrin.

Fenitrothion technical (128% w/v), Bayer (Australia) Pty Ltd, is active against a variety of adult insects and is readily available.

Fenvalerate is a synthetic pyrethroid registered for use against Heliothis larvae, in cotton, as Sumicidin ULV (active ingredient: fenvalerate 40 g/l), Shell Chemical (Australia) Pty Ltd. Fenvalerate has previously been used for adult bioassay with some success (Gunning, pers. comm.). It is the lowest in activity of the synthetic pyrethroids used against Heliothis spp. and less toxic to handle. This quality was important because the spraying technique used necessitated a large amount of handling of the chemical.

Cypermethrin is the most active of the synthetic pyrethroids against the larvae of Heliothis spp. (Edge, pers. comm.). The high toxicity was considered an advantage because the moths were covered in hairs and

scales which could have prevented all, or a proportion, of the chemical which impinged on the insect from being absorbed.

Unfortunately the cypermethrin was difficult to obtain and a quantity was obtained only after the number of moths in the field population had declined. A sufficient number of moths could not be obtained to conduct trials with all three insecticides. So cypermethrin was omitted from the study.

1.2 AIMS

(a) To choose, from fenitrothion and fenvalerate, the chemical with the highest effectiveness against adults of Heliothis spp..

(b) To establish the lethal dose (LD50) for the chosen chemical against adults of Heliothis spp.

1.3 METHODS AND MATERIALS

1.3.1 Time and location of the experiments

The moths were trapped and the bioassay carried out at the New South Wales Department of Agriculture Research Station at Trangie in northwestern New South Wales. The experiments were run from 10/5/85 to 20/5/85 inclusive, during the time when the Heliothis spp. activity is usually high.

1.3.2 Moths

The moths for the bioassay were caught in traps, rather than raised in a laboratory culture, because laboratory facilities were unavailable at the time of the experiments and the time available for this study was limited.

The moths were trapped at night over irrigated lucerne which was in flower. The average crop height was 45 cm with approximately 80% ground cover. Texas pheromone traps were used (Plate 1.1) as these were more efficient than the traditional funnel trap (Gregg, pers. comm.) and enabled the moths to be caught alive and undamaged. To allow for any changes in wind direction overnight, the traps were placed in the centre of the crop and erected so that the entrance to each trap, and the pheromone lure, were level with the top of the canopy. The traps were emptied each morning at 0730 h, before the temperature rose above the limits of tolerance of the moths. The moths were dosed on the day of capture.

1.3.3 Rangefinding tests

Rangefinding tests, or short-term bioassays, were carried out to establish which of the insecticides, fenitrothion and fenvalerate, was most active against Heliothis spp., and to determine the approximate range of doses to be used in the definitive trials. The upper limit was to be at 85% mortality and the lower limit at 15% mortality.

One percent stock solutions of Sumicidin technical (fenvalerate) in acetone and fenitrothion technical in acetone were made and refrigerated for use throughout the trials. The same stock solution was used throughout to reduce errors in dilution. Serial dilutions of this base stock solution were made using acetone and a "control" treatment of acetone only was included in each trial.

The concentrations used for the initial rangefinding tests were:

1.0%, .1%, .01%, .001%, .0001% and acetone.

The first two catches of moths were predominantly H. punctigera and H. armigera respectively. Trials were run using fenitrothion with 5 insects per dose (Table 1.1). The moths from the next catch were H. punctigera, which were dosed with fenvalerate. The lower dose limit was not established in this trial, but as expected, the chemical appeared more active than fenitrothion. The next catch was again principally of moths of H. punctigera. As it seemed that subsequent catches were likely to be of moths of H. punctigera, and that the field population was necessarily of limited duration, it was decided to continue using fenvalerate and to subject the moths from each night's catch to a wide range of doses in the hope of identifying the lower limit, as well as producing replicates of dose response.

The doses used were the logarithmic series:

0.2%, 0.1%, 0.05%, 0.025%, 0.0125%, 0.00625%, 0.00312%, 0.00156%, 0.00078%, 0.00039%, 0.00019%, 0.000095%.

After treatment the moths were transferred to 4 litre, clear plastic containers. Each container had holes drilled in the lid and also on the side 2 cm from the base. Moisture was provided by a 2 * 1 cm block of blue household sponge soaked in a 10% glucose solution. A maximum of 10 moths were kept in a container.

Knockdown and mortality were recorded at 6, 12, 24, 36 and 48 hours after topical application. Knockdown was defined as the "apparent death" of a moth immediately after exposure to the insecticide followed by recovery. An insect was considered to be dead when it could no longer right itself after being tipped onto its back.

1.3.4 Dosing technique

Each moth was dosed by placing a 1 microlitre (µl) droplet just behind the head using a Burkard Arnold Hand Microapplicator (Rickmansworth Herts. England) fitted with an Agla Micrometer all-glass syringe and a 30 g hypodermic syringe needle. The needle had been shortened by cutting at right angles and filed, then bent downwards at an angle of 45 degrees to facilitate application. The applicator had a button-stop mechanism to enable accurate delivery of 1 µl droplets.

The moths were pacified using carbon dioxide. Initially forceps were used to pick up the moths but it was found that many fluttered violently and damaged themselves badly. It was discovered that a moth would readily step onto a piece of filter paper when the paper was placed in front of it and pressed gently against it's front legs. Once on the paper they could be easily positioned under the applicator needle and

usually remained calm during dosing and transfer to their relevant containers.

Dosing was carried out at the same temperature (27° +/-2° C) and time of day. Light conditions were kept constant throughout the experiment.

As the total number of insects per catch was limited, only 10 insects were used per dose. The number of moths in the field population dropped considerably before the third replicate could be carried out so that only 20 insects were subjected to each dose.

1.3.5 Analysis

Analysis was carried out with the technique suggested by Busvine (1971) for calculation of the log dose/probit regression line.

No knockdown was observed in the trials so the analysis was carried out with the 24 hour mortality data. Death after this time could well have been due, partly at least, to the result of natural ageing of the field population.

1.4 RESULTS

1.4.1 Rangefinding tests

The mortality data from the rangefinding trials using fenitrothion and fenvalerate against H. punctigera and H. armigera (Table 1.1) suggested that the fenvalerate was more active against the moths of H. punctigera. At 24 hours, only 50% mortality was achieved at the highest dose of

fenitrothion compared with 100% for fenvalerate. The 30% mortality which occurred at 0.01% fenvalerate after 6 hours may have been caused by handling rather than the chemical because no mortality was recorded for the other doses.

1.4.2 Later tests: calculation of LD50

(LD50 is defined as the lethal dose that will kill 50% of a susceptible population (Rand, 1980)).

The regression equation for the dose response line can be accepted only if the calculated improved expected probit (Y') values corresponding to the original values of x do not differ from the expected probit (Y) by more than 0.2 (Busvine, 1971). The initial transformation of dose-mortality data (Table 1.2) produced differences greater than 0.2 in the four highest doses. To correct for this the values of the working probit (y) were used as the improved expected probit Y' and the calculations repeated.

The corrected mortality (Table 1.2) was calculated using Abbot's formula:

$$Pt = \frac{Po - Pc}{100 - Pc} * 100$$

where Pt = corrected mortality %

Pc = control mortality %

Po = observed mortality

The equation of the log dose/probit line of best fit was calculated using regression analysis (Appendix 1) on the revised parameters (Table 1.2).

The equation calculated was:

$$y = 5.282 + 0.6579(x - 2.574) \qquad (1)$$

The LD50 can be calculated by substitution in equation (1) such that:

at the LD50 $y = 5$

therefore $x = \frac{6.694 - 5.282}{0.6579}$

$= 2.145$

Thus the LD50 occurs at log dose 2.145. Since the log doses were transformed by adding 5 to make them positive, the LD50 occurs at the untransformed log dose -2.855. This dose is equivalent to a concentration 0.0014%.

The amount of fenvalerate in a 1 µl droplet can now be estimated.

Given that 1 ml of a 1% solution contains: $1.199 * 10^{-3}$ g/ml

1 ml of a 0.0014% solution contains: $1.678 * 10^{-6}$ g/ml

which is equivalent to: 1.678 µg/ml

the dose applied in a 1 µl droplet = 0.0016786 g.

To now express this amount as a function of body weight: if a moth weighs 0.082 g, at the LD50 the amount of fenvalerate in µg per g of body weight of moth

$$= \frac{0.0016786}{0.082}$$

$$= 2.046 * 10^{-2} \text{ µg/g body weight}$$

The transformation of the dose-mortality data and the regression analysis were carried out using two programmes written for the Apple IIE computer (Appendix 1).

1.4.3 Calculation of the precision of the LD50

In order to estimate the precision of the LD50, the variance and Chi squared value for homogeneity of data were calculated. The calculated Chi squared is usually compared with the tabulated value at 5% probability. If the calculated value, exceeds the tabulated value heterogeneity is indicated. The degrees of freedom for the Chi squared

value are n-2, where n = number of replicates. As only 2 replicates could be completed the degrees of freedom would be 2-2 = 0 which is invalid. The observed Chi squared was 30.1492 which is well in excess of any tabulated values in the range of degrees of freedom which would normally be used.

Let the LD50 log dose 2.20 = m

Then the variance of m =

$$v = \frac{1}{b^2} * \left(1 + \frac{(m - xbar)}{Sw\ Swx^2 - \frac{(Swx)^2}{Sw}}\right)$$

$$= 2.3095 * \left(0.0094 + \frac{0.1391}{93.4549}\right)$$

$$= 2.51 * 10^{-2}$$

Where:

sw = 106.12

swx = 273.119533

swy = 560.4992

swx^2 = 796.376521

swy^2 = 3031.01814

swyx = 1504.03053

xbar = 2.57368576

ybar = 5.28174897

b = 0.657891521

From the Chi squared test for homogeneity of data:

$$X^2 = (Swy^2 = ybar * Swy) - b * (Swxy - xbar * Swy)$$

$$= 70.6295 - 40.4803$$

$$= 30.1492$$

Degrees of freedom = number of experiments - 2

$$= 2 - 2 = 0$$

1.5 DISCUSSION

The scattered nature of the points about the regression line indicate a high degree of heterogeneity in the results which is borne out by the excessively high Chi squared value. The heterogeneity was probably brought about by a number of factors leading to a variable response to the doses. For example:

(i) the moths were drawn directly from a field population so that the age of the individuals was unknown and likely to be variable.

(ii) The nutritional status of the insects was not known. It is possible that the insects developed on different food sources because Heliothis are found in a number of host crops, many of which were grown in the Trangie district at the time. Heliothis are also highly mobile insects which are known to

migrate between crops within regions and between regions. It is possible, therefore, that each night's catch did not come from precisely the same source area. The variation in type and quality of the larval diet could cause differences in their physiology which could lead, in turn, to differing responses to the insecticide and to the carbon dioxide used as a pacifier. The different nutritional backgrounds may have caused variations in body weight which would have altered response to the insecticide.

To get an estimate of the variation in body weight, twenty males were weighed and found to be relatively uniform in weight, having a mean body weight of 0.082 +/- s.e. 0.0045 g.

(iii) The carbon dioxide, used as a pacifier, could have altered the response of the insects to the insecticide.

(iv) The incidence of disease in a field collected population is unknown, as is the occurrence of parasitism. If a diseased moth is dosed, it could die from the effect of the disease rather than that of the insecticide. The mortality as a result of disease could distort the mortality caused by the insecticide. Hopefully the mortality caused by factors other than the insecticide would become evident in the control.

(v) Variation in the moths' responses to fenvalerate may have been increased by the thick covering of scales and hairs over their bodies. These scales and hairs may have prevented the complete

dose in each microlitre droplet from contacting the epidermis and being absorbed. The droplet did appear to spread on contact with the moth, rather than remain on the surface. Ideally, removing hairs from the dosing point would have avoided this problem, but the moths were difficult to handle and susceptible to handling damage.

Finally, the small sample size would have been a source of variation also enhancing the effects of the other variables. It is widely accepted that 30 insects per dose is a minimum number (FAO 1969, 1970). Only 20 insects, and in some cases only 10 per dose were used so that heterogeneity in the results is to be expected.

Although it was necessary to choose a chemical that would be active against the moths, this bioassay has served to demonstrate a technique rather than give a recommended dose for field application. Dose response data from bioassays using topical application cannot be directly applied to conventional field application of insecticides, because insects in the field obtain an unknown dose either by direct impingement of the chemical on the insect, ingestion, contamination from dosed vegetation or a combination of these. It is not possible, therefore to ensure that an insect will receive the specified dose emitted. Chemical is also lost through drift and breakdown in the environment so that, if the LD50 dose were to be applied to an insect population in the field, a 50% kill is highly unlikely to be achieved.

Flying insects are more efficient collectors of insecticide than settled insects (Spillman, 1976); hence it is likely that the kill achieved if

an LD50 were to be applied to flying targets would be closer to the 50% mark than if applied to settled targets. As a result, provided the spray cloud consisted of mainly small droplets, one would expect to be able to use less chemical when spraying flying insects because it is being used more effectively.

PLATE 1.1: A Texas Pheromone Trap Set up in Irrigated Lucerne, Trangie N.S.W.

Figure 1.1. Original and improved dose response lines for H. punctigera treated with fenvalerate.

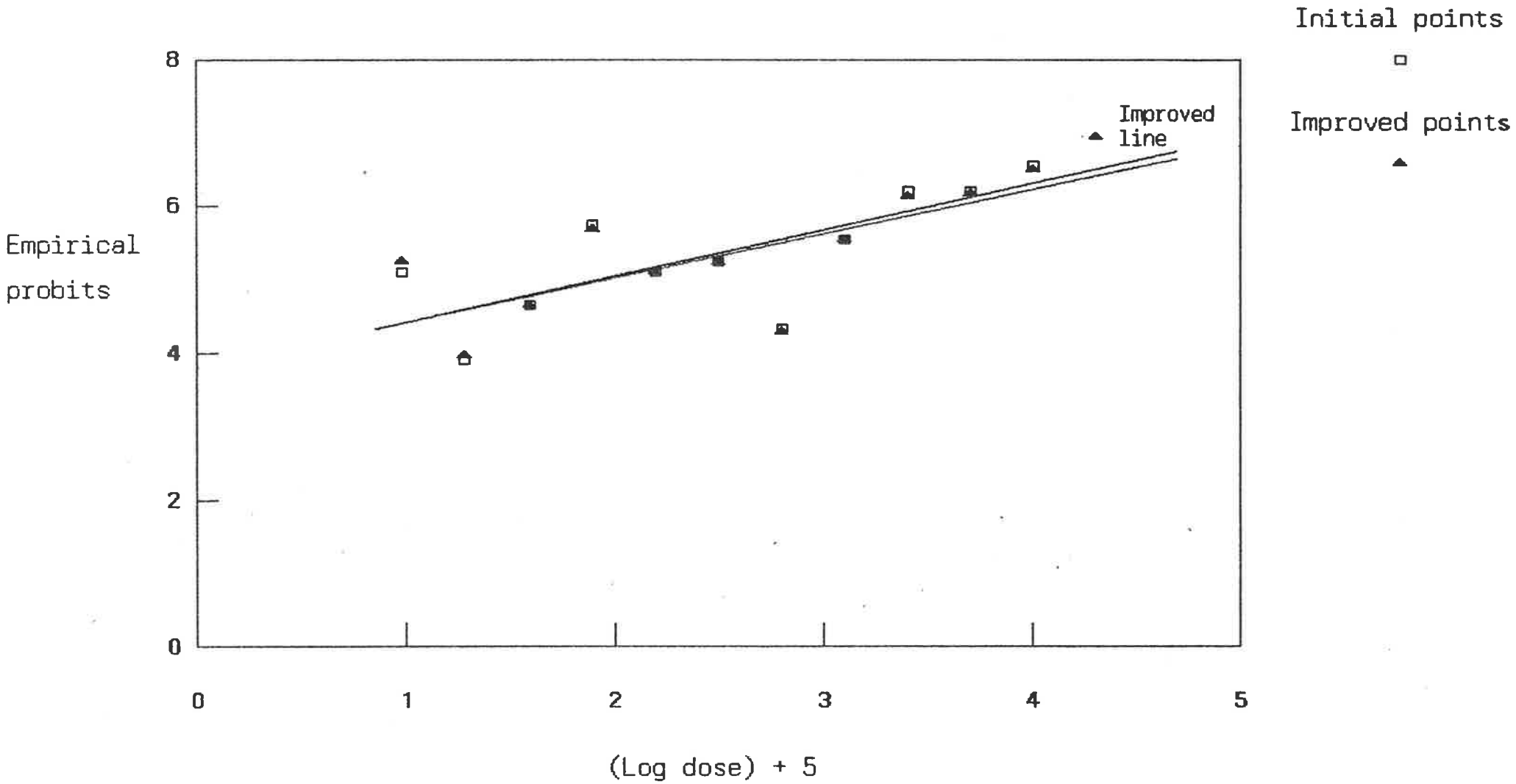


TABLE 1.1: Mortality of adults of H. punctigera and H. armigera in rangefinding tests using fenitrothion and fenvalerate technical diluted in acetone

		H. punctigera			H. armigera		
Pesticide Concentration (%)	HRS after treatment (h)	Number tested	Number dead	Corrected mortality (%)	Number tested	Number dead	Corrected mortality (%)
Fenitrothion							
0.0	6	10	0	-	10	1	-
0.0001		5	0	-	5	0	-
0.001		5	0	-	5	2	30
0.01		5	0	-	5	0	-
0.1		5	0	-	5	0	-
1.0		5	0	-	5	0	-
0.0	12	10	0	-	10	1	-
0.0001		5	0	-	5	1	11
0.001		5	0	-	5	2	30
0.01		5	1	20	5	1	11
0.1		5	1	20	5	2	30
1.0		5	1	20	5	1	11
0.0	24	10	2	1	10	1	-
0.0001		5	1	0	5	1	11
0.001		5	2	25	5	3	55
0.01		5	3	50	5	1	11
0.1		5	2	25	5	4	77
1.0		5	3	50	5	4	77
Fenvalerate							
0.0	6	10	1	-			
0.0001		5	0	-			
0.001		5	2	30			
0.01		5	0	-			
0.1		5	0	-			
1.0		5	0	-			
0.0	12	10	1	-			
0.0001		5	1	11			
0.001		5	2	30			
0.01		5	1	11			
0.1		5	2	30			
1.0		5	1	11			
0.0	24	10	1	-			
0.0001		5	2	30			
0.001		5	3	55			
0.01		5	2	30			
0.1		5	4	77			
1.0		5	5	100			

TABLE 1.2 **Parameters used for calculation of the log dose/probit line for H. punctigera exposed to a series of doses of technical fenvalerate by topical application**

Fenvalerate concentration %	No. insects used	No. dead (24 hrs)	% dead	Corrected % mortality	Log dose (+5) x	Empirical probit	Expected probit Y	Working probit y	Improved expected probit Y'	Second improved probit Y'
0.000	30	4	13.3							
.000095	10	6	60.0	53.86	.9777	5.10	4.02	5.56	4.2541	4.2318
.00019	20	5	25.0	13.49	1.2787	3.90	4.25	3.96	4.4474	4.4298
.00039	20	9	45.0	36.56	1.5910	4.65	4.55	4.65	4.6480	4.6357
.00078	10	8	80.0	76.93	1.8920	5.74	4.80	5.68	4.8413	4.8333
.00156	10	6	60.0	53.86	2.1931	5.10	5.02	5.10	5.0346	5.0314
.00312	20	13	65.00	59.63	2.4941	5.24	5.20	5.24	5.2279	5.2294
.00625	20	7	35.00	25.02	2.7958	4.33	5.52	4.25	5.4217	5.4279
.0125	20	15	75.0	71.16	3.0969	5.55	5.79	5.53	5.6151	5.6260
.025	20	18	90.0	88.46	3.3979	6.20	6.02	6.15	5.8084	5.8240
.05	20	18	90.0	88.46	3.6989	6.20	6.30	6.19	6.0017	6.0220
.1	20	19	95.0	94.23	4.0000	6.55	6.52	6.56	6.1950	6.2201
.2	10	10	100.0	100.00	4.3010	-	6.65	7.17	6.3884	6.4181

Chapter 2

SPRAY TRIALS

2.1 INTRODUCTION

One of the main aims of the study was to determine whether a chemical insecticide could be drifted over a cotton canopy at night, so that it formed a uniform blanket over the crop.

For the cloud to remain aloft and drift, very small droplets (vmd up to 50 μm) are required. Spillman (1976) maintains that, for most flying insects, the probability of droplets being caught by an insect is maximized when droplets are in the range vmd 10-30 μm. No conventional spraying device is capable of producing a monosized droplet spectrum; however, the spinning disc atomizer (MicronULVA) chosen for use in these trials is capable of producing a cloud with the mode at the lower end of the spectrum for given revolutions per minute (rpm) and flow rate (Spillman, 1980).

For the insecticidal cloud to be effective against flying moths it must be maintained for a number of hours whilst the moths are flying. Both H. punctigera and H. armigera have a bimodal pattern of night flight activity with the peaks occurring 2-3 hours after sunset and before sunrise (Wardaugh et al., 1976). The females of both species are most active in the early evening, so spraying was timed to cover this peak of activity. Night observations that were made before the trials began confirmed that the moths did fly at the evening time suggested by Wardaugh.

The study also aimed to investigate whether the insecticide cloud produced in the manner described would kill H. punctigera and H. armigera moths. The cloud of small droplets should stay aloft and drift downwind, hopefully creating a barrier of chemical at the flying height of the moths, which would persist for some time. A moth need only fly through the cloud once to obtain enough insecticide to kill it, so the technique does not rely on a moth flying for a long time or having to fly more than once during a spray period. A number of insect sampling schemes were carried out to determine the effect of spraying on the Heliothis population.

2.2 METHODS AND MATERIALS

(i) Location and time of trials

The trials were conducted in northwestern N.S.W. on the Nevertire property of Auscott Pty Ltd. The trials ran for 6 consecutive nights from 30/1/85 to 5/2/85 inclusive - a time in the cotton growing season when the incidence of Heliothis spp. is usually high.

The areas chosen for the trial and control plots were fields 1000 m * 600 m and 900 m * 200 m respectively, on the outskirts of the main cotton growing area of the property. Both were surrounded by graded roads and had fallow or stubble areas off to two sides. The location was chosen so that the plots would be as far as possible from any spraying that might have been done against Heliothis on other cotton fields.

The control plot remained unsprayed but was sampled for adults and eggs in the same way as the sprayed plot.

The crop, (irrigated cotton var. Delta Pine 61), was supporting flowers, squares and bolls and had a mean height of 68 +/- s.e. 1.14 cm. The canopy was closed within the rows but not between rows.

The synthetic pyrethroid Sumicidin ULV (Active ingredient: fenvalerate 40 g/l) Shell Chemical (Australia) Pty Ltd was sprayed over the trial plot following the bioassay results.

(ii) The spray rig

A MicronULVA (Micron Sprayers Ltd, Three Mills, Bromyard, Herefordshire) was mounted on a frame on the back of a Toyota Landcruiser Utility 3.5 m above the ground. In previous trials by Rendell (1980) an uneven distribution of insecticide across the crop was achieved with a sprayer mounted at a fixed point in a cotton field. By mounting the sprayer on the back of a vehicle, thereby making it mobile, it was hoped to improve the uniformity of spray cover. The frame was made of welded rolled hollow steel (RHS) to provide as firm a base as possible in order to reduce pulsation of the sprayer while the vehicle was moving over rough ground (Plate 2.1).

The sprayer was fitted with the smallest cone attachment, to keep flow rate and droplet size to a minimum, and connected to a 12 volt battery and rheostat. The sprayer was operated at 13,000 rpm which is the maximum practical operating speed which gives a relatively narrow

droplet spectrum with a vmd around 30 μm (Symmons, pers. comm.). The rpm was checked before each trial using a stroboscope.

Chemical was fed to the sprayer from a standard 500 ml conical MicronULVA bottle which screwed vertically into the MicronULVA head.

(iii) Spraying the crop

The crop was sprayed from the upwind edge/edges, with the vehicle set at (750 rpm), for 6 consecutive nights. Each night's spray trial began at 2100 h and continued for at least two hours to cover the period when the moths should have been flying (pers. obs., Wardaugh et al., 1976). When the direction of drift was at right angles to one of the sides of the plot, the spray rig travelled only along that one upwind side. Two passes of the vehicle constituted one spray run. If the direction of drift was other than at right angles, the rig travelled along both upwind sides to ensure an even cover of spray across the crop. A run, in this case, consisted of a single pass along each upwind side.

The cloud of insecticide was constantly renewed by spraying continuously during an extended period when the moths were flying.

The amount of chemical lost from the cloud depends chiefly upon the wind speed and the turbulence. The trials were conducted as far as possible under inversion conditions to minimize the effects of turbulence and maintain the cloud for as long as possible.

The temperature was recorded using a whirling psychrometer, wind direction with smoke flares and windspeed with a direct reading cup anemometer. Recordings were made at the beginning and end of the spray period and whenever a wind change was observed during spraying. Throughout spraying the average windspeed was recorded at 27 cm, 59 cm, 122 cm and 248 cm above the base of the furrow within the crop, with a stack of cup anemometers mounted on a tower.

(iv) Sampling for insecticide deposits

A grid of whirling samplers (Plate 2.3) strung with white "Milford" knitting cotton (4 ply) and strips of Kromecote paper was set out in the 600 m * 1000 m trial plot (Fig. 2.1). Each sampler was powered by a 12 volt battery and set to spin with a peripheral velocity of c.5 m/s. The samplers were strung with fresh cotton thread and Kromecote paper before each trial. The cotton threads were collected and frozen after spraying and the samplers and scissors washed with acetone to prevent cross contamination. The fenvalerate content of the cottons was determined using high pressure liquid chromatography (HPLC). The Kromecote papers were used as a cross check for the presence of insecticide droplets at the sampler site should problems have arisen in the detection of fenvalerate by the HPLC.

(v) Sampling the Heliothis population

The Heliothis population was monitored in the test and control paddocks before the trials began and each day throughout the trials.

Two of the standard funnel type pheromone traps used by SIRATAC Ltd cotton management system, one for each Heliothis species, were placed in the control and test paddocks following each trial. The traps were checked and emptied each evening at 1700 h.

In order to estimate the number of adults present in the crop, 4 transects on foot were done in each plot. The cotton was planted in rows a metre apart with approximately 1 plant per metre. To make walking easier the transect lines followed the furrow between the rows. The cotton bushes on either side of the furrow were beaten with a stick and the number of moths which rose from these two rows was counted. Because the change in population, rather than absolute numbers, was the variable being estimated, the same transect lines were followed each day. Any changes which may have occurred would not then have been a function of changed location.

The transects were begun at 1800 h when the moths appeared more easily disturbed than they were earlier in the day (pers. obs.). In the sprayed plot the transects were 600 m long running along the rows from the northern to the southern edges of the crop. Recordings of moth numbers were made over intervals of 100 m to determine whether or not there were any gradients in the moth distribution. The transects were spaced at 300 m intervals across the plot leaving a buffer zone of 50 m at either end to eliminate edge effects. The control plot was smaller; the transects were 200 m long from the eastern to the western edges of the crop and spaced at 100 m, again leaving a 50 m buffer zone. Recordings were made over 50 m intervals.

Egg counts were carried out each day using the stratified random sampling method employed by SIRATAC. After leaving a buffer zone, by walking 75 m into the crop, five plants were counted in a row and the next five were then examined. The counter then moved on another five plants and across seven rows and began again repeating the process until 60 plants had been examined. Thirty plants were examined, beginning from the north east corner of the plot, and another 30 beginning at the south west corner. The terminals of each 5 plants in the top 12 cm of the canopy were examined and egg numbers recorded.

Light trap and pheromone trap catches for both the immediate area of the test plots and for the region were obtained from Auscott to give an overall picture of the Heliothis population in the area.

2.3 RESULTS

(i) Windspeed during each trial

The estimated windspeed during each trial is given in Table 2.1. It was calculated from the rpm readings of the anemometer at the 1.22 m position on the stack of anemometers. The value given is the mean windspeed during the spray period.

TABLE 2.1: The windspeed, duration of spraying, amount of insecticide applied and estimated flow rate in each trial

Trial	Average windspeed at 122 cm (m/s)	Duration of spraying (min.)	Chemical applied (ml)	Flow rate (mls/min.)
1	0.176	98	1199	10.9
2	1.380	84	870	10.35
3	2.318	78	965	10.96
4	0.857	128	1270	9.84
5	0.336	116	1320	10.31
6	0.815	124	1210	9.76

Trial 1 began under inversion conditions. The direction of drift moved from SSW to SE after the first spray run and remained steady throughout the rest of the spraying period. The runs were changed accordingly (Fig. 2.2).

Trial 2, which is shown to have had an average windspeed of 1.38 m/s in Table 2.1, also began under inversion conditions with a 0.5 m/s breeze registering on the hand-held anemometer. The inversion broke towards the end of the spray period when a windspeed of 1-2 m/s was shown on the hand-held anemometer during the last 30 minutes of spraying. The changes in direction of drift were similar to those in Trial 1: the drift remained almost due south for most of the period. Hence, most of the runs were done along the southern border.

On the night of Trail 3 there was no inversion and the breeze was a steady easterly enabling spray runs to be done on one side of the plot only (Fig. 2.2).

Trial 4 is shown to have had an average windspeed of only 0.857 m/s. However, there was no inversion on this night and a strong 4-5 m/s breeze sprang up in the last 10 minutes of the spray period. The wind direction was from the north east throughout the trial so there was no change in the position of the spray runs.

Trial 5 also began under inversion with the direction of drift predominantly from the north west. A shift to the south west occurred 30 minutes before the end of spraying.

In Trial 6 the change in direction of drift came half way through the spray period. This trial also began under inversion conditions.

(ii) The numbers of moths

The moth counts given in Table 2.2 were the numbers seen per 100 m of each of the 4 transects. Since the cotton plants were spaced at approximately one per metre along the rows, the number of moths per 100 m is effectively the number of moths per 200 plants, because moths were counted from each row on either side of the transect.

The counts were low throughout the trial period, as also were egg counts (Table 2.3) and pheromone trap catches of moths (Table 2.4).

The average pheromone catch over the region was obtained from counts collected under the SIRATAC scheme from cotton properties in the Macquarie irrigation district. These catches covered a 10 day period which included the time when the trials were underway. The mean daily catches, although low, were more than double those for H. punctigera trapped in the trial and control plots. For H. armigera the mean catch was slightly less than double the catch in the sprayed plot and more than double the catch in the control plot.

The pheromone catches were predominantly H. armigera, which was expected for that time of the season. H. punctigera is usually regarded as a spring pest.

By contrast the light trap catches (Table 2.5) contained more H. punctigera than H. armigera. The light trap was not located at the spray site and was used only to get an idea of the amount of moth activity in the general area.

(iii) Chemical recovery

The chemical recoveries at the sample sites were variable. A fuller discussion of the recoveries is given in Chapter 3.

2.4 DISCUSSION

Ideally the aim of spraying is to control the insecticide cloud such that the desired concentration reaches a target area and is maintained there for a sufficient period so as to cause the death of the insect. Spraying must be timed at the period when the insects are "available"

for control (e.g., the morning or evening periods of flight activity of the Heliothis moths) so that maximum use is made of the emmitted chemical. Any droplets which do not reach their target are essentially environmental pollution. A number of questions are therefore raised which these preliminary trials do not attempt to answer; such as the duration of flight for an individual moth, the height to which the moths fly, the frequency of flight in an evening and the percentage of the population which fly per night. All of these questions affect the method and effectiveness of the spray technique. For these trials it was assumed that the whole population would not be flying at any one time and that an individual may not fly every night. Hence spraying was carried out for several hours to cover the evening peak of moth activity and for 6 nights to cover the major period of a population peak in the field.

The numbers obtained from the transects for moths (Table 2.2) and the egg counts (Table 2.3) were too sparse to enable conclusions to be drawn regarding the effect of spraying on the moth population, especially since there are likely to be inherent variations in the moth population within and between the paddocks (Wardaugh et al., 1980, Wardaugh, pers. comm.). The low numbers make it difficult to reasonably compare the sprayed and unsprayed paddocks each night and over time and conclude whether changes in total numbers and distribution are due to the effects of spraying or due to natural variation. The low numbers also make it difficult to ascertain whether the chemical had a cumulative effect i.e., moths dying after receiving a number of sublethal doses which may have appeared as an increasing kill over time.

Little is known of the sublethal effects of fenvalerate on Heliothis distribution. The chance that an uneven cover of chemical over the crop may have simply moved the moths within the plot cannot be examined.

PLATE 2.1: Spray Rig Used for the Trials

PLATE 2.2: Spinning Disc Atomiser - Micronulva Used on the Spray Rig

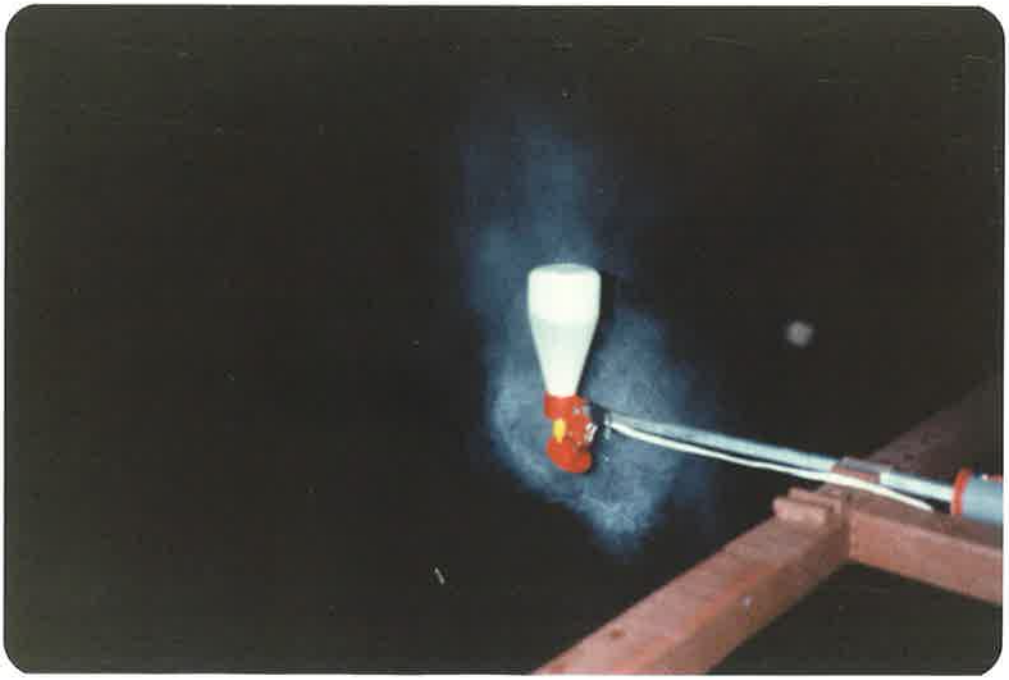


PLATE 2.3: A Whirling Sampler Strung with Cotton Thread Used for Sampling the Insecticide Cloud

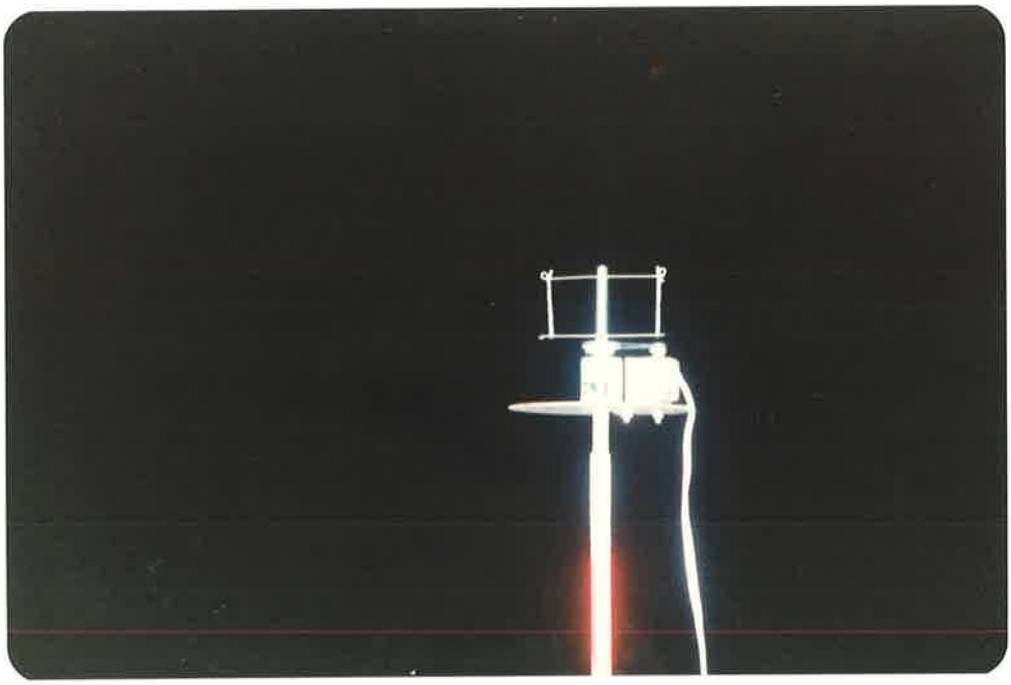


Figure 2.1. Sampler layout, dimensions and surrounding environment of the trial plot.

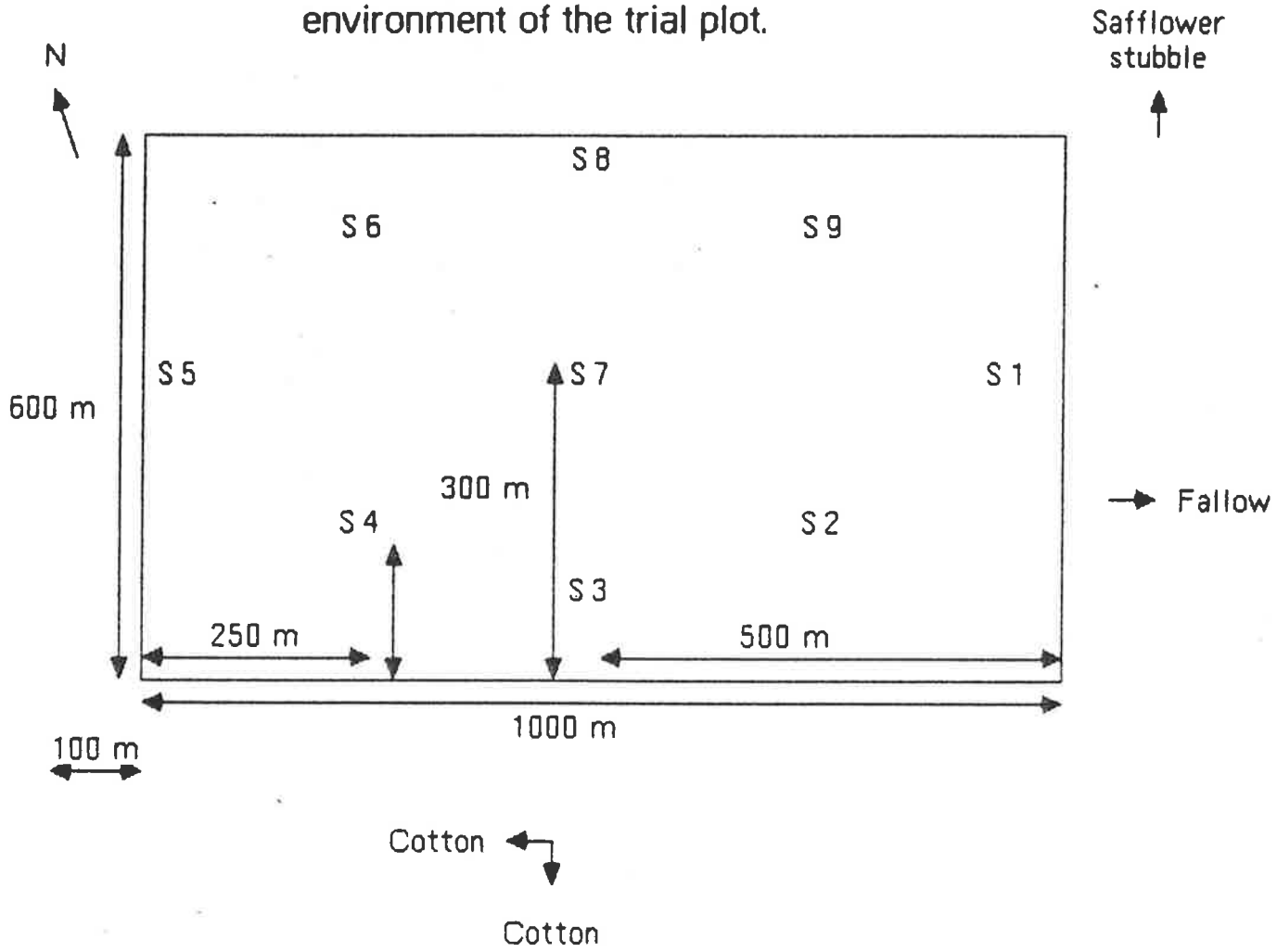


Figure 2.2. Layout of samplers, direction of wind, track of spray vehicle and amount of chemical collected at each sampler (ug/cm^2) for trials 1 to 6.

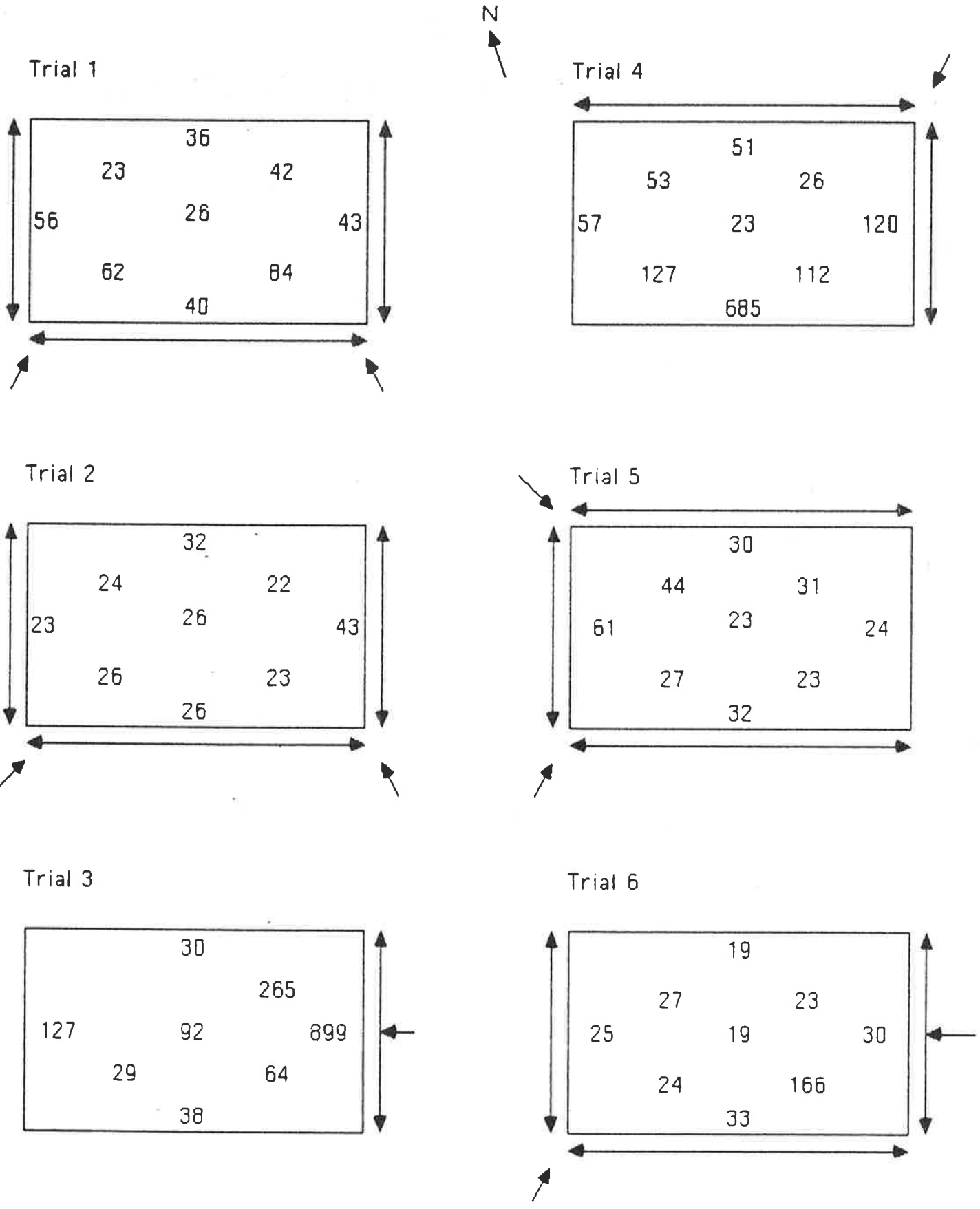


TABLE 2.2: Daily counts of Heliothis adults per 100 m of foot transect in the sprayed and control plots

Date and transect no.		4 x 600 m transects Sprayed plot						4 x 200 m transects Control plot			
		100	200	300	400	500	600	50	100	150	200
29/1	1	0	0	0	0	0	0	0	1	0	0
	2	0	1	0	0	0	0	0	0	1	0
	3	0	1	1	0	0	0	0	0	0	0
	4	0	0	1	0	0	1	1	1	0	0
Total							5				4
30/1	1	0	1	0	0	1	2	0	1	0	0
	2	0	0	0	0	1	0	0	0	1	1
	3	0	0	0	0	0	0	0	0	0	0
	4	0	1	0	0	0	0	0	0	0	0
Total							6				3
31/1	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	1	0	0	0	0	0
	4	0	0	0	1	0	0	0	0	0	0
Total							2				0
1/2	1	0	0	3	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	2	1	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
Total							6				0
2/2	1	1	1	0	2	0	0	0	0	0	0
	2	1	1	0	2	0	0	0	0	0	0
	3	0	0	1	1	0	1	0	0	0	0
	4	0	1	0	0	0	1	0	1	0	0
Total							13				1
3/2	1	0	0	0	0	0	0	0	0	0	0
	2	0	2	0	2	1	1	0	0	0	0
	3	1	1	0	0	1	1	0	0	0	0
	4	1	1	2	1	0	0	0	0	0	0
Total							15				0
4/2	1	0	0	0	0	0	1	0	0	0	2
	2	0	0	0	0	2	1	1	1	0	0
	3	0	2	2	0	2	1	1	0	1	1
	4	3	6	2	1	2	1	0	0	0	0
Total							26				5
5/2	1	0	0	1	0	0	0	0	0	1	0
	2	0	0	1	0	2	1	1	1	0	0
	3	0	2	1	0	1	0	1	0	0	0
	4	0	0	0	0	1	0	0	0	0	0
Total							10				4
Σ		7	22	16	10	15	12	5	6	4	4
$\bar{x}$		1.16	3.66	2.66	1.66	2.50	2.00	0.83	1.00	0.66	0.66

TABLE 2.3: Number of eggs counted per 5 cotton plants on the terminals extending into the top 12 cm of crop canopy. Counts were done 2 days prior to spraying and each day following a treatment.

Date of Sampling		No. of eggs per sub-group of 5 plants												Σ	$\bar{x}$
29/1	S	0	1	0	0	0	0	1	0	0	0	0	0	2	.033
	C	0	0	1	0	0	0	0	0	0	1	0	0	2	.033
30/1*	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31/1	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/2	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2/2	S	0	1	1	1	0	0	0	0	1	0	0	0	4	.066
	C	0	1	0	0	1	0	0	0	0	1	0	0	3	.050
3/2	S	0	0	0	1	1	1	0	0	1	0	0	0	4	.066
	C	0	0	2	0	0	1	0	0	1	1	0	0	5	.083
4/2	S	0	0	1	0	0	0	0	1	0	1	0	0	3	.050
	C	0	1	0	0	0	1	0	0	1	0	0	0	3	.050
5/2	S	0	1	0	0	2	0	0	0	2	0	1	0	6	.100
	C	1	1	0	0	0	1	0	0	0	0	0	0	3	.050

S : Sprayed plot
C : Control plot

* First night of spray trials

TABLE 2.4: Pheromone trap catches of adult male Heliothis from the control and sprayed paddocks. Traps were placed each night after spraying and removed before spraying commenced on the following evening.

Dates of counts	Sprayed Paddock		Control Paddock	
	H. punctigera	H. armigera	H. punctigera	H. armigera
29/1	0	0	2	0
*30/1	0	2	0	0
31/1	0	2	0	0
1/2	0	5	0	0
2/2	0	0	0	1
3/2	0	1	0	0
4/2	1	0	0	1
Σ	1.00	10.0	2.00	2.00
$\bar{x}$	0.13	1.42	0.27	0.27

* First night of spray trials.

AVERAGE TRAP CATCH PER DAY FOR THE MACQUARIE DISTRICT OVER THE 10 DAYS UP UNTIL 5/2/85:

H. punctigera : 3.31 moths

H. armigera : 2.37 moths

TABLE 2.5: Light trap catches of H. punctigera and H. armigera for one night prior to the commencement of spraying and each night of spraying

Date	H. punctigera	H. armigera
29/1	26	0
*30/1	15	0
31/1	14	2
1/2	9	0
2/2	8	1
3/2	38	0
4/2	43	7
Σ	153	10
$\bar{x}$	21.86	1.43

* First night of spray trials.

Chapter 3

FENVALERATE ANALYSIS USING HIGH PRESSURE LIQUID CHROMATOGRAPHY

3.1 ESTABLISHING THE METHOD FOR DETECTING FENVALERATE USING HPLC

3.1.1 Introduction

The HPLC comprises:

- pump: Waters M - 45 Solvent Delivery System
- injector: Waters Universal Liquid Chromatograph Injector Model U6K
- column: Altex Ultrasphere liquid chromatography column Model 256-06
- flow cell: Altex Model 155
- spectrophotometer: Hitachi Model 155 with power supply
- integrator: Hewlett Packard 3390A

It operates by separating the components of a sample within the column and releasing them at different times. The spectrophotemeter, set at a particular wavelength, then identifies each component and the resulting signal is recorded in graph form by the integrator as a series of peaks along a time axis. The integrator calculates the area under each peak, and from this the amount of chemical injected, and in the sample, can be calculated.

As each chemical has its own specific characteristics it will be retained for a specific time, and thus the peak on the graph which represents it will also occur at a specific time. The time can be

varied by the composition of the solvent used in the system and its flow rate. Hence a number of standards must be run to establish (a) the time at which a peak appears, (b) the best solvent mix to be used and (c) the optimum flow rate.

3.1.2 Materials and methods

A 1% stock solution of fenvalerate technical in methanol (methyl alcohol CH_3OH) was made. The fenvalerate did not dissolve readily in methanol so it was dissolved initially in 0.5 ml of acetone.

A 0.01% solution was used for the analysis so that the peak would be large in comparison with those for the solvent, methanol and acetone. Acetone and methanol were also injected to identify where they appeared on the printout.

The standard solvent for analysis of fenvalerate technical is a mixture of acetonitrile CH_3CN) and milli Q water (Ferris, pers. comm.). After a series of test runs involving trial and error, changing the solvent mix and the flow rate, the ratio of 75% acetonitrile and 25% milli Q water with a flow rate of 2 ml/min. was decided upon. This combination caused the fenvalerate peak to appear at approximately 9 minutes. Increasing the concentration of acetonitrile shortened the time taken for the fenvalerate peak to appear, but brought it close to the solvent peaks; undesirable, because of the possibility of the peak being masked by the solvent peaks, if the retention time is affected by other factors, such as changes in room or solvent temperature.

3.2 DEVELOPING A STANDARD CURVE FOR FENVALERATE

To calculate the amount of fenvalerate injected into the HPLC and hence in each sample, a calibration curve must be developed. The curve gives the relationship between the area under the fenvalerate peak (Fig. 3.1) and a known series of concentrations which have been injected.

A series of 5 dilutions was made from the stock solution namely: 0.1%, 0.05%, 0.025%, 0.00125%, 0.000625%; and 20 µl of each was injected. Two replicate injections were made and the resulting mean areas plotted against the amount of fenvalerate (µg). The relationship was linear (Fig. 3.2). The equation of the line and hence the slope 'b' and intercept 'a' pertaining to fenvalerate could be calculated using linear regression.

The equation $y = a + bx$

where x = area under the peak (units)

y = amount of fenvalerate injected (µg)

b = slope

a = y intercept

Once the constants were known, the amount of fenvalerate injected from a sample could be calculated by substitution into the equation of the regression line.

The fenvalerate content of the sample in µg/cm can then be calculated as follows:

$$\frac{H * I * J}{K * L * M}$$

where H = amount of fenvalerate injected (µg)

I = amount of eluent to remove fenvalerate from sep-pak (ml)

J = amount of solvent used for extraction (ml)

K = amount injected into HPLC (ml)

L = area/weight of sample to be soaked (cm^2 or g)

M = amount of extract to go through the sep-pak.

3.2.1 Results

Calculation of the fenvalerate content of the injected samples used for developing the standard curve was carried out as follows:

Density of fenvalerate = 1.199 g/ml

Amount of fenvalerate in 1 ml of a 1% solution = $1.199 * 10^{-2}$

therefore:

0.1% solution has $1.199 * 10^{-3}$ g

0.05% solution has $5.990 * 10^{-4}$ g

0.025% solution has $2.990 * 10^{-4}$ g

0.0125% solution has $1.495 * 10^{-4}$ g

0.00625% solution has $7.47 * 10^{-5}$ g

therefore:

1 ml of a 0.1% solution has 1199 µg

1 ml of a 0.05% solution has 599 µg

1 ml of a 0.025% solution has 299 µg

1 ml of a 0.0125% solution has 149.5 µg

1 ml of a 0.00625% solution has 74.75 µg

The amount of fenvalerate in 20 µl of each sample:

0.1% .....23.98 µg

0.05% .....11.98 µg

0.025% .....5.98 µg

0.0125% .....2.99 µg

0.00625% .....1.495 µg

Using the information from Table 3.1 the equation of the calibration line of best fit was calculated by regression where:

x = the area under the peak

y = the amount injected in µg

The equation of the line of best fit is:

$$y = 0.1657997 + (5.300979 * 10^{-5} * x) \qquad (1)$$

The calibration line obtained for fenvalerate is shown in Fig. 3.1.

The coordinates of the line of best fit obtained by substitution in this equation were:

$x = 0, \qquad y = 0.1657997$

$x = 416890, \qquad y = 22.26505$

The correlation coefficient = 0.99919 shows a strongly linear relationship between the points.

Using equation (1), the amount of fenvalerate injected into the HPLC (y) for all subsequent samples could be calculated by substitution (Section 3.3).

The theory of regression analysis indicates that the regression line should pass through the origin of the x and y axes (0,0). The equation of the line would then be $y = ax$. The HPLC has a level of sensitivity below which it is unable to detect the presence of a chemical and the level of sensitivity is specific for that chemical. Thus, a reading of zero area given by the HPLC may not mean that there is no chemical present.

The y intercept 'a' is therefore a measure of sensitivity of the instrument. The regression line, obtained from the calibration of the HPLC for fenvalerate, had a y intercept 'a' = 0.1657997. This value indicated that when a reading of zero area was reported, the sample in fact contained 0.1657997 µg of fenvalerate. The y intercept was

included in the equation, expressed in the form y = a + bx, as a correction factor.

The mean area under the fenvalerate peak, and the corresponding concentrations of the 20 µl samples injected from the standard solutions of fenvalerate, are given in Table 3.1.

3.3 HPLC ANALYSIS FOR FENVALERATE FROM SPRAYED COTTONS

3.3.1 Sample preparation and clarification

The frozen cotton threads were thawed in the jars in which they were collected (1 per jar). Five millilitres (mls) of methanol - the solvent used to extract the fenvalerate from the threads - were added to each jar and left for 12 hours in the dark.

Four mls of extract were pipetted into a test tube and made up to 20 mls with milli Q water.

The fenvalerate was separated from the other components in the extract by passing the 20 mls of extract through a SEP-PAK C18 cartridge attached to a hypodermic syringe. The liquid was forced through the SEP-PAK which separated and collected the various classes of components in the sample.

The fenvalerate is then removed by eluting with 2 mls of methanol giving a sample ready for injection. Two replicates, each 20 µl, were injected from each sample and the fenvalerate content of each sample calculated.

The HPLC was operated at a flow rate of 2 mls/min., 240 nm and range 1.0.

3.4 RESULTS

The fenvalerate recoveries are shown in Table 3.2 along with their means and standard errors; they were calculated with a computer programme written for the purpose (Appendix 2).

The recovery from Trial 3 sampler 6 (T3 S6) is missing because analysis of both replicate cottons had been carried out before it was discovered that the HPLC was faulty.

Trial 2 had the most uniform distribution, three of the nine samplers having 26 µg/cm and two having 23 µg/cm. The difference between the highest and lowest recoveries was 13 µg as opposed to 870 g in Trial 3.

The HPLC is a sensitive machine and readings can vary with temperature changes in the solvent and injected sample as well as slight electricity fluctuations. To ensure the area obtained had not been affected by these factors two replicate injections were made from each sample. The areas under the fenvalerate peaks, shown in Table 3.2, are the mean of the areas obtained for the two replicates from each sample. The area obtained for each replicate is shown in Appendix 3.

The variation between samplers for each trial was determined by calculating the relatively variability of the recoveries:

$$\frac{\text{standard error of the mean}}{\text{mean recovery of fenvalerate}}$$

(Table 3.3). The relative variabilities have been expressed in graph form (Fig. 3.3) by plotting the mean fenvalerate recovery for each trial against the corresponding standard error of the mean. Trials 1,2,5 and 6 had similar recoveries and the relative variabilities of Trials 1,2 and 5 were less than 20%. In Trials 3,4 and 6 there were samplers which had extremely high recoveries (Table 3.2) in comparison with other samplers in each Trial, e.g. T3 S1, which would have caused the higher standard errors.

3.4.1 Calculation of the approximate amount of chemical available to a moth

If:

Plot size = 60 ha

Mean output of insecticide per night = 1139 ml.

Then:

Amount of insecticide per hectare = 18.98 ml.

If:

Duration of flight of a moth is c.3 seconds

Collecting surface area of the moth is c.1 sq. cm

The moth flies at c.3 m/s

Then:

The moth will fly through 9 metres and have a collecting surface of 0.9 sq metres.

The amount of chemical available over 1 sq. metre:

$$= \frac{18.98}{10000}$$

$$= 0.001898 \text{ ml}$$

The amount of chemical available over 0.9 sq. metre = 0.0017082 ml.

The active ingredient in 1 ml of the chemical sprayed = 0.04 g.

Therefore the amount of active ingredient that could be collected by a moth making one 3 second pass through the spray cloud is:

$$= 75.92 \text{ } \mu g$$

Given that the LD50 is 0.2047 µg/g body weight and that a moth weighs 0.082 g, a moth collecting only half as efficiently as the whirling samplers would receive a lethal dose.

3.5 DISCUSSION

3.5.1 Chemical recovery

In any field trials dealing with biological systems, the number of variables is always high. Although as many factors per trial as

possible were kept constant in this study, and despite relatively constant weather conditions, differences still occurred. The main difference was due to the change in direction of drift causing changes in position of the spray runs. It was not physically possible to run replicate trials on the same night because it was very difficult to find identical paddocks and the time and manpower involved was prohibitive.

The fenvalerate recoveries from Trials 1,2,5 and 6 were similar from sampler to sampler and between trials, evident from the relatively small standard deviation from the mean of the recoveries for each night (Table 3.2). The similarities and differences between the recoveries for the 6 Trials are emphasized in Fig. 3.3. The recoveries for Trials 3 and 4 were very different from those of the other four Trials. Some of the samplers appeared to have received large amounts of insecticide and others had recoveries similar to those in Trials 1,2,5 and 6. The third and fourth Trials were conducted under conditions of light wind in contrast to 1,2,5 and 6 each of which was conducted under inversion for the most part.

There are a number of factors which could explain the large variation in chemical recovery in Trials 3 and 4 as well as the smaller variations in Trials 1,2,5 and 6.

(i) Contamination

The first of these was the possible contamination of the cottons during collection. Extreme care was taken to ensure the samplers and scissors were washed after removing each set of cottons from their sampler. The

amount of insecticide on the cottons would have been very small, so that even the smallest amount on hands or clothing, coming in contact with the cottons, would have caused a large difference in readings.

It is possible that some of the samplers may have accumulated more chemical than others through the position of the spray runs and the direction of drift, however for those isolated samplers with excessively high readings e.g. T3 S1, T4 S3 and T6 S2, contamination may have increased the fenvalerate recovery.

(ii) Characteristics of the ground

The roughness of the ground could have been a second important factor contributing to atmospheric turbulence and hence the random movement of droplets within a spray cloud. Small droplets travel at the speed of the air surrounding them and are carried by the parcel of air in which they are released. Turbulence over cotton is greater than over bare ground, the air movement being affected by changes in crop height, as well as the furrows between the rows, over which the canopy had not closed. Even under inversion conditions, such as experienced in Trials 1,2,5 and 6, the turbulence is minimized but not eliminated (Spillman, 1970). The surface of the crop would still have affected the movement of the spray cloud, possibly channelling it away from or towards the samplers depending upon the characteristics of the surface around the sampler site.

(iii) The nature of eddy turbulence

Thirdly, the direction of the eddies created by turbulence is random and droplets may be moved upwards and downwards, so that the concentration of insecticide in a given parcel of air above the crop may vary from place to place within the plot. The insecticide recovered from the sampler will reflect the concentration of chemical in the air at the sampler site. These random changes in concentration of insecticide in the atmosphere could also account for the variation in recovery. However, as the movement of the eddies is random, one would hope that constant renewal of the spray cloud would result in a uniform total recovery for the whole spray period from each sampler.

(iv) Changes in atmospheric conditions

A fourth factor leading to variations in insecticide recovery could have been the change in atmospheric conditions experienced in Trials 1,2,5 and 6. The Trials began under inversion conditions and the insecticide droplets could be seen hanging in the air above the crop when followed with a spotlight. Although the larger droplets would have sedimented out in the early stages following release, many of the smallest droplets may have been dispersed upwards and left hanging in the atmosphere unable to go beyond the top of the inversion layer. The distribution of these droplets is unlikely to be uniform throughout the inversion layer above the crop because of the factors associated with random movement of air parcels already discussed.

In all four trials a slight increase in wind speed, breaking the inversion, was recorded on the hand held anemometes towards the end of spraying. The sudden associated changes in atmospheric conditions - reduced temperature and increased turbulence - may have caused 'dumping' of the localized pockets of suspended droplets from the inversion layer which could have created an uneven pattern of insecticide recovery across the grid of samplers. e.g., T5 S5 61 µg which is approximately twice that of 7 of the 8 other samplers.

(v) The effect of wind

Trials 3 and 4 were not carried out under inversion conditions. The spray period for Trial 3 started with a 0.5 - 1 m/s breeze which increased to 3 - 4 m/s 40 minutes before the end of spraying. The wind direction was consistently easterly and the spray run on the eastern edge was along the bank of the irrigation channel which was 2 m above the level of the crop and the other spray runs. The deposits at the samplers nearest the spray run, across the centre and at the far edge were the highest. The reading of 899 µg/cm at S1 was extremely high with respect to the others in the paddock and is one which may have been affected by contamination. However, it is possible that the bank along which the run was driven, being at right angles to the wind, caused a large amount of turbulence as the wind left the top of the channel dragging the spray cloud down towards the crop increasing impaction on the sampler causing a chemical recovery reading.

Trial 4 was conducted under a steady 1 - 1.5 m/s breeze though the average speed for the spray period shows less than 1 m/s. There appears

to be a division of the spray deposit between the SE and NW halves of the paddock (Fig. 2.2). This may have been the result of the NE wind drifting the spray diagonally towards the southern boundary on the eastern run and the western boundary on the northern run. The sprayer was switched off when travelling from the eastern to the northern track because the irregular ground at the corner and relatively still conditions, as well as change in the direction of travel of the vehicle meant, the people in the back of the vehicle would have been covered in spray. The effect of switching off the sprayer may have been to reduce the amount of spray reaching S7 and S9 while the furthest sampler of the diagonal, S4, would have collected spray from both sides.

Eddy diffusion produced in unstable windy conditions moves droplets both up and down. The result may be an increase in depth of a small droplet cloud and therefore a decrease in the concentration at any one point within the cloud. The turbulence is likely to make the concentration uneven across the crop.

3.5.2 Chemical recovery with respect to LD50

Although the recoveries were variable none were below the estimated LD50 for fenvalerate. The approximate amount of chemical that would have been available to a moth, on an average during each trial, was well in excess of the lethal dose required to kill 50% of the population. If the moths were only half as efficient at collecting the chemical as were the whirling samplers, the dose received would have been lethal to a substantial portion of the population.

Insecticides affect insects in 2 ways - either by direct toxic effects causing mortality or by sublethal effects when the dose arriving at the insecticidal site of action in the insect is not enough to cause death (Kamlesh et al., 1984). Sublethal doses have been shown to cause latent toxicity, enzyme induction, stimulatory and inhibitory effects on reproduction, altered behaviour and physiology. A suggestion has been made recently, that sublethal effects of pyrethroids such as fenvalerate are important in crop production because of their antifeedant and repellent activities (Kamlesh et al., 1984). For example, the sublethal concentrations of fenvalerate LC1 and LC50 (the concentrations lethal to 1% and 50% of the population respectively), had a deleterious effect on larval development and reduced adult longevity and fecundity of the diamondback moth Plutella xylostella (L.). In addition, the female moths preferred to oviposit on leaves that remained untreated (Kamlesh et al., 1984).

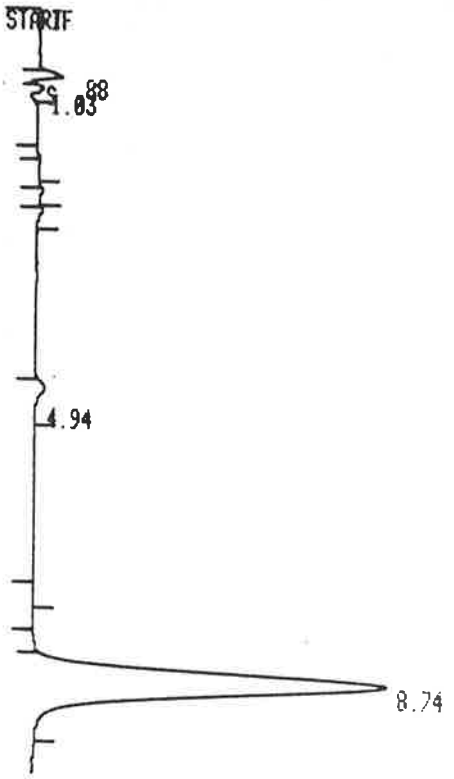
Given the variable recovery between samplers and the relatively large distance between the samplers, it is possible that there were areas of sublethal concentration within the plot and certainly outside the plot where drift would have occurred. Some chemical would have doubtless sedimented onto the crop in both lethal and sub-lethal concentrations.

Unfortunately the moth population was not high enough to detect changes in the population distribution or patterns of egg lay as a result of spraying, so it was not possible to tell whether fenvalerate had the same effect on Heliothis spp. as P. xylostella. Should the repellent and antifeedant properties hold true for Heliothis spp., many moths may

be driven from the treated cotton field into the adjacent ones, giving a false impression of the success of control within the treated paddock.

Although the chemical recoveries from the samplers were variable, the distribution of the insecticide appeared to have produced levels which would be toxic to moths at a much lower application rate - 18.98 ml/ha - than that recommended for control of larvae up to 2 cm long - 1.5 - 3.5 l/ha.

Figure 3.1. A printout from the Reporting Integrator of the HPLC, showing the fenvalerate peak recorded from an injection of 20 µl from a solution of 0.025 % fenvalerate in methanol.



RUN # 3 APR/03/85 17:37:36

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.88	2077	SBB	0.037	1.920
1.03	878	BB	0.088	0.812
4.94	1774	BB	0.204	1.640
8.74	103460	BB	0.336	95.629

TOTAL AREA= 108190
MUL FACTOR= 1.0000E+00

Figure 3.2. Calibration line for fenvalerate

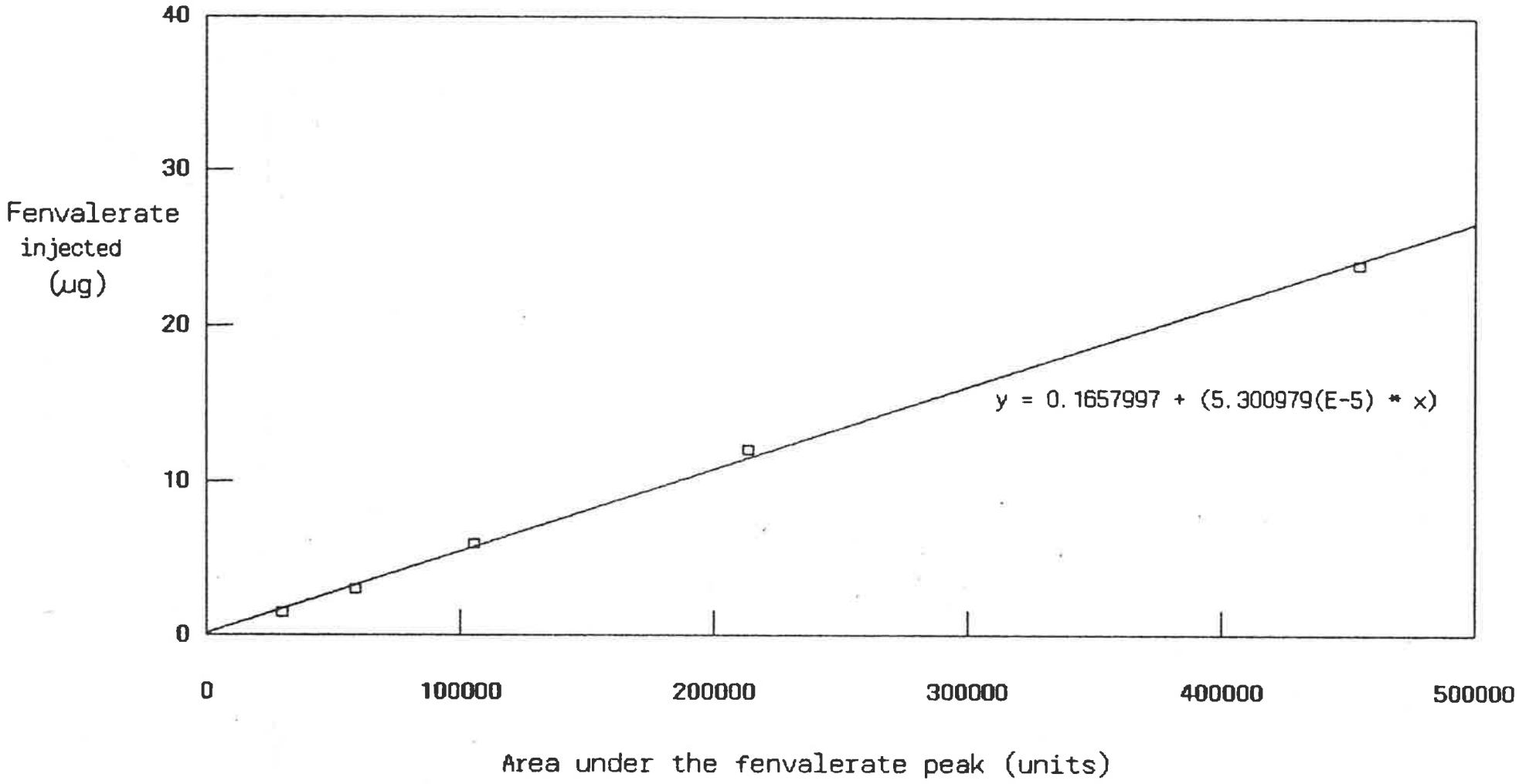


Figure 3.3. The relative variability of the amounts of fenvalerate recovered from samplers in each trial.

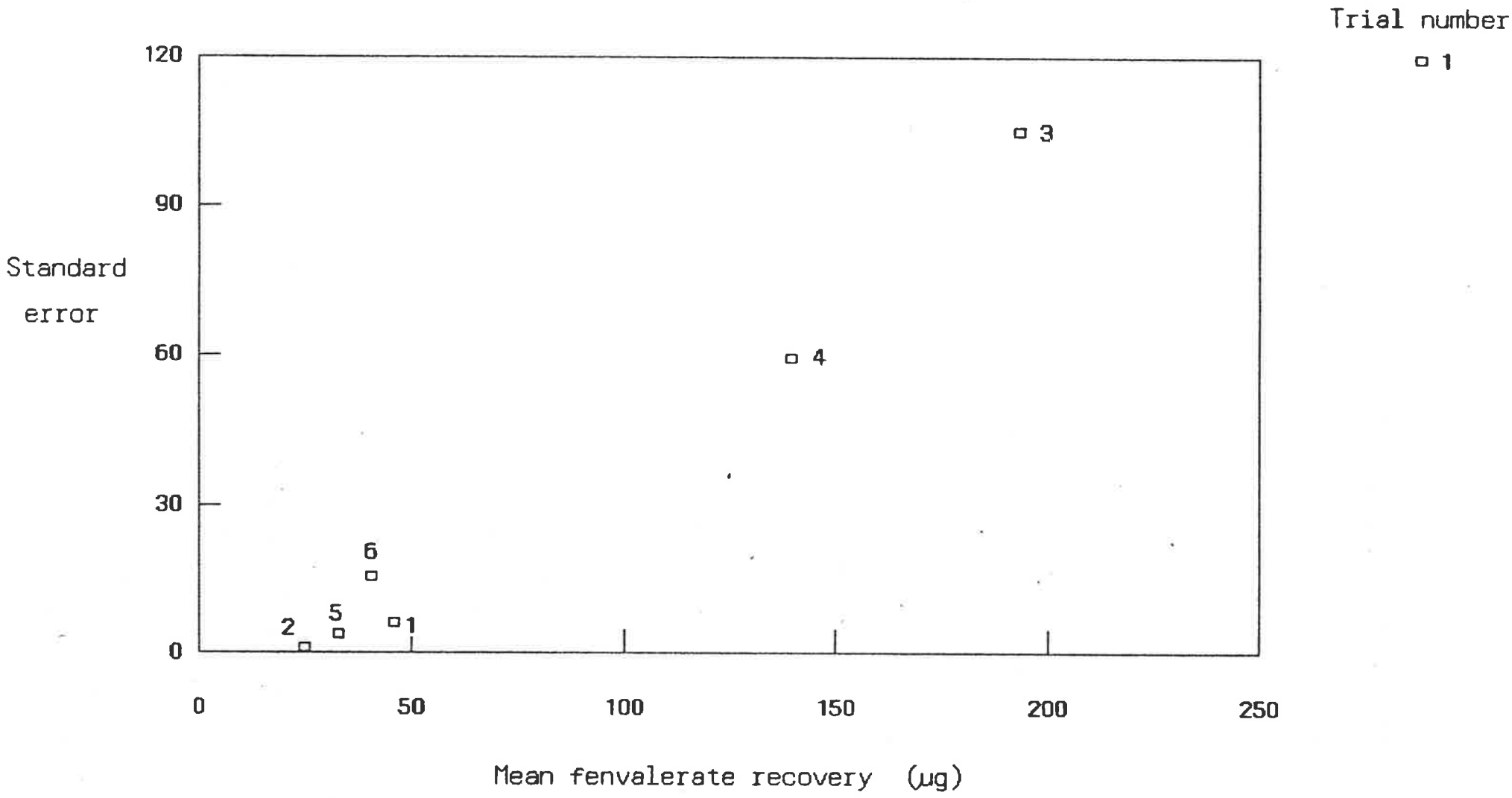


TABLE 3.1: Area under the fenvalerate peak for replicate injections of 20 µl samples from standard solutions used to construct the standard curve (Fig. 3.2); The mean of these areas and the corresponding fenvalerate concentration (µg)

Fenvalerate concentration in standard solutions %	Area under replicate 1 (units)	Fenvalerate peak replicate 2 (units)	Mean area under fenvalerate peak (units)	Amount of fenvalerate injected (µg)
0.1	491180	416890	454035	23.98
0.05	204000	221660	212830	11.98
0.025	106860	103460	105160	5.98
0.0125	46459	59146	58343.5	2.99
0.00625	28719	30830	29774.5	1.495

TABLE 3.2: Areas under the fenvalerate peaks at calculated by the reporting integrator of the HPLC, corresponding quantity of fenvalerate recovered from the cotton thread on each sampler and length of cotton thread

Trial (T) and Sample (S)	Length of cotton (cm)	Mean area under peak (units)	Fenval. content of sample ($\mu g/cm^2$)	Trial and sample no.	Length of cotton (cm)	Mean area under peak (units)	Fenval. content of sample ($\mu g/cm^2$)
T1 S1	10.5	3621	42.59	T4 S1	11.5	17655	119.75
2	11.5	11424	83.85	2	14.5	21404	112.11
3	11.5	3860	40.26	3	13.5	136330	684.50
4	11.0	7096	61.80	4	14.0	23715	127.05
5	10.5	5982	55.94	5	11.5	6707	56.67
6	11.5	820	23.03	6	12.0	6554	53.46
7	11.5	1349	26.41	7	12.5	1117	22.50
8	9.5	1996	35.74	8	10.0	4586	51.11
9	9.5	2820	41.49	9	11.0	1257.5	26.42
Mean			45.67	Mean			139.29
Standard error			6.30	Standard error			69.41
T2 S1	13.5	771	19.14	T5 S1	12.0	1268.5	24.28
2	11.5	909	23.26	2	14.0	1632	22.53
3	11.0	1244	26.33	3	14.0	3658.5	32.12
4	11.5	1348	25.79	4	13.5	2284.5	26.57
5	12.5	1294	23.44	5	11.0	7002.5	61.02
6	12.5	1441	24.22	6	13.0	5789.5	43.77
7	12.0	1535	25.75	7	13.0	1459.5	23.38
8	9.5	1461	32.01	8	10.0	1341.5	29.61
9	14.5	1582	21.52	9	10.5	1732.5	30.67
Mean			24.61	Mean			32.66
Standard error			1.19	Standard error			4.14
T3 S1	13.5	159750	899.39	T6 S1	11.5	1993.5	29.51
2	12.5	9892	63.91	2	10.5	23185.5	166.05
3	14.0	4309.5	37.91	3	12.0	2892	33.24
4	18.0	2945.5	28.75	4	12.5	1420.5	24.11
5	12.5	20862	127.17	5	11.5	1177	24.80
6				6	10.0	871	26.50
7	11.0	12107.5	91.78	7	13.0	591	18.96
8	12.0	2354.5	30.27	8	12.5	366.5	18.52
9	11.5	42940.5	265.44	9	10.5	539.5	23.27
Mean			193.08	Mean			40.55
Standard error			104.94	Standard error			15.76

TABLE 3.3: The mean, standard error of the mean, relative variability and percent relative variability of the amount of fenvalerate recovered from each trial

Trial number	Mean fenvalerate recovery (μg)	Standard error	Relative variability	% relative variability
1	45.67	6.30	.138	25.37
2	24.61	1.19	.048	8.82
3	193.08	104.94	.544	100.00
4	139.29	69.41	.498	91.54
5	32.66	4.14	.127	23.35
6	40.55	15.76	.389	71.51

Chapter 4

DISCUSSION

Of the hypotheses proposed for study at the outset of this thesis, only the first two have been tested satisfactorily and have had a positive outcome.

These were:

1. That a spray cloud consisting of small monosized droplets will remain aloft at a given height and across a specified area of cotton.

2. That the chemical remaining aloft will be sufficient to kill adults of Heliothis spp. across the entire field.

The spray trials have shown that chemical can be drifted across one kilometre of crop, and possibly further, under conditions of inversion and light winds at concentrations capable of killing Heliothis spp. adults. The nine whirling samplers served to give an overall impression of the insecticide distribution across the crop - one that appeared to be relatively uniform when spraying under inversion but quite variable when spraying was conducted in light winds. The variation in recovery, at points which were widespread over the plot, indicates a need for more samplers per unit area to enable a clearer picture to be drawn of the distribution and recovery of insecticide over the crop. It would have been useful to have had three replicate collections at a given distance

from the spray run - the diamond grid pattern gave only one sample in some cases (Fig. 2.1). Five rows of three samplers, evenly spaced across the plot, would have given the three replicates. An increase in the number of samplers would have enabled unusually high or low recoveries, such as that for T3 S1, to be checked.

The trials must be extended and repeated, with more samplers per unit area and replicate trials on each night. The vegetation should be sampled for sedimentation of chemical and the surrounding paddocks sampled for drift. When planning subsequent trials it will be necessary to take into account the difficulties associated with night work - the labour intensive nature of the sampling and spraying, the cost of the trials and the precision required in the results.

The third and fourth hypotheses pertained to the Heliothis population. They were:

3. That the adult population within the area sprayed will be suppressed.

4. That the egg lay will be reduced and hence the larval population be suppressed.

The aim was to spray on consecutive nights during a seasonal peak of moth activity. The 1984-85 growing season was very dry prior to the trials and there had been little insect activity throughout the Macquarie district. The trials were conducted in the field which had experienced the highest insect pressure throughout the season, on the

Nevertire property of Auscott Pty Ltd. Nevertheless, the light trap, pheromone trap and egg counts showed very low Heliothis numbers for the month prior to the trials, as well as during and after spraying. Unfortunately, the lack of moths prevented assessment of the technique for killing moths in the field, or assessing it's effect on oviposition or the distribution of moths.

Should subsequent trials show that the method is successful in killing adult Heliothis in flight after dark, a chemical must be chosen which is specific to the moths. As drift is the prime objective, some of the chemical will end up over adjacent land. It is essential that any beneficial Lepidopterous insects within and outside the crop should not be affected. If the chemical is not specific to the adults, the larvae may be subjected to sublethal doses which sediment onto the vegetation. Repeated application of sublethal doses could cause selection for resistance in the larvae which could extend through to the adult stage.

The spraying technique, as it stands is not meant to be used by the landholder; however, this study has provided valuable groundwork for further study on a principle of spraying which, if successful, could have a major effect on the method of controlling Heliothis and other insects.

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APPENDIX 1

CALCULATION OF THE REGRESSION LINE RELATING PROBITS AND LOG DOSE

The two programmes listed in this appendix were written to transform the dose-mortality data obtained in the bioassay described in Chapter 1 and to calculate the regression line relating probits and log dose using the method suggested by Busvine (1971).

PROGRAMME TO CALCULATE EXPECTED PROBITS FROM DOSE - MORTALITY DATA

```
10 HOME
20 PRINT "EXPECTED PROBIT CALCULATION"
30 PRINT
40 INPUT "HOW MANY DOSE LEVELS";Z
45 PRINT
60 PRINT
70 DIM B(20),Y(20),W(20)
80 DIM X(20),K(20),N(20)
85 DIM P(20),Q(20),L(20)
88 DIM M(20),NY(20),E(20)
89 DIM 0(20)
100 FOR I = 1 TO Z
110 PRINT "DOSE ";I
115 PRINT : INPUT "No. OF INSECTS USED= ";0(I)
116 PRINT
120 INPUT "LOG DOSE = ";X(I)
130 PRINT : INPUT "EXPECTED PROBIT = ";Y(I)
140 PRINT : INPUT "WORKING PROBIT = ";B(I)
150 PRINT : INPUT "WEIGHTING COEFFICIENT = ";K(I)
160 PRINT : PRINT : PRINT : NEXT I
170 HOME
190 PRINT
225 PRINT :
226 PR#1
230 PRINT "OLD Y        NEW Y"
```

```
240 FOR I = 1 TO Z
250 W(I) = K(I) * O(I)
260 W = W + W(I)
270 N(I) = W(I) * X(I)
280 N = N + N(I)
290 P(I) = W(I) * B(I)
300 P = P + P(I)
310 Q(I) = N(I) * X(I)
320 Q = Q + Q(I)
330 L(I) = B(I) * P(I)
340 L = L + L(I)
350 M(I) = P(I) * X(I)
360 M = M + M(I)
361 NEXT I
370 XB = N/W
380 YB = P/W
390 B = (M - (XB * P)) / (Q - (XB *N))
395 FOR I = 1 TO Z
400 NY(I) = (YB - (B * XB) + (B * X(I)))
401 NEXT I
405 FOR I = 1 TO Z
407 PRINT : PRINT
408 NY (I) = INT (NY(I) * 10 ^ 4 + .5) / INT (10 ^ 4 + .5)
410 PRINT TAB( 3);Y(I);TAB(12);NY(I)
419 NEXT I
420 PRINT : PRINT
430 PRINT "SW = ";W
440 PRINT "SWX = ";N
450 PRINT "SWY = ";P
460 PRINT "SWX2 = ";Q
```

```
470 PRINT "SWY2 = ";L
480 PRINT "SWYX = ";M
490 PRINT "XBAR = ";XB
500 PRINT "YBAR = ";YB
510 PRINT "b = ";B
520 PRINT : PRINT " W         WX          WY"
530 FOR I = 1 TO Z
540 PRINT
550 PRINT TAB( 2);W(I); TAB( 10);N(I); TAB( 18);P(I);
560 PRINT : NEXT I
570 PR#0
```

PROGRAMME TO CALCULATE THE REGRESSION EQUATION USING DATA FROM THE CALCULATION OF EXPECTED PROBITS

```
10 HOME
20 INPUT "YBAR = ";YBAR
30 PRINT
40 INPUT "XBAR = ";XBAR
50 PRINT
60 INPUT "b = ";B
70 PRINT
80 INPUT "X = ";X
90 Y = YBAR - (B * XBAR) + (B * X)
100 PRINT : PRINT "FOR X = ";X;"Y = ";Y
105 PRINT : PRINT
110 GOTO 80
```

APPENDIX 2

CALCULATION OF FENVALERATE RECOVERY ON COTTON THREADS

The programme listed here calculates the amount of fenvalerate per square centimetre of cotton thread recovered from a sample analysed by HPLC. The programme converts the areas under the fenvalerate peak as given by the HPLC to concentrations using the equation of a standard curve for fenvalerate (Chapter 3).

PROGRAMME TO CALCULATE FENVALERATE CONTENT OF COTTONS USING HPLC

```
10 HOME
15 PRINT : PRINT : PRINT : PRINT : PRINT : PRINT:
20 PRINT "PROGRAM TO CALCULATE FENVALERATE"
30 PRINT
40 PRINT "  CONTENT OF COTTONS USING HPLC"
45 PRINT : PRINT : PRINT : PRINT :
51 FOR N = 1 TO 50
52 N = N + 1
53 NEXT N
60 HOME
65 INVERSE
66 PRINT "PARAMETER INPUTS"
67 PRINT : PRINT : PRINT :
68 NORMAL
80 PRINT
90 PRINT "AREA OF COTTON SOAKED IN SOLVENT (cm2)"
100 INPUT A
110 PRINT
120 PRINT "AMOUNT OF SOLVENT USED FOR SOAKING (ml)"
130 INPUT B
140 PRINT
150 PRINT "AMT OF EXTRACT THROUGH SEP - PAK (ml)"
160 INPUT C
170 PRINT
180 PRINT "AMT OF METHANOL TO WASH SEP - PAK (ml)"
190 INPUT D
200 PRINT
```

```
210 PRINT "AMOUNT INJECTED INTO HPLC (ml)"
220 INPUT E
221 PRINT
225 PRINT "IDENTIFICATION OS SAMPLE"
226 INPUT A$
230 PRINT
240 PRINT "AREA UNDER PEAK (units)"
250 INPUT F
260 PRINT
270 G = (0.00005300979 * F) + 0.16579966
280 H = (G * D * B) / (E * C * A)
285 H = INT(H * 10 ^ 4 + .5) / INT (10 ^ 4 + .5)
286 G = INT(G * 10 ^ 4 + .5) / INT (10 ^ 4 + .5)
290 PRINT "PRINTED RESULTS ? (Y/N)"
300 INPUT Z$
305 HOME
310 IF Z$ = "Y" THEN GOSUB 8000
311 PRINT : PRINT : PRINT : PRINT
400 PRINT "SAMPLE NAME";A$
410 PRINT
420 PRINT "AMOUNT OF FENVALERATE INJECTED ";G;" ug"
430 PRINT
440 PRINT "FENVALERATE CONTENT OF SAMPLE ";H
445 PRINT "ug PER cm2 OF COTTON"
450 PRINT
451 PRINT "----------------------------------------"
460 PR#0
470 PRINT : PRINT : PRINT : PRINT
480 INVERSE
6000 PRINT "ALL NEW PARAMETERS OR JUST AREA"
```

```
6100 PRINT
6200 PRINT "Y FOR ALL NEW, A FOR AREA"
6300 INPUT B$
6310 NORMAL
6320 HOME
6400 IF B$ = "Y" THEN GOTO 60
6410 IF B$ = "A" THEN GOTO 225
7900 END
8000 PR#1
8100 RETURN
```

APPENDIX 3

THE AREA UNDER THE FENVALERATE PEAK OBTAINED FROM EACH REPLICATE INJECTION (a,b) OF EXTRACT FROM THE COTTON THREAD ON EACH SAMPLER

Trial no. (T)	replicates (a,b)	Sampler number 1	2	3	4	5	6	7	8	9
T1	a	3610	11050	3875	7131	5737	903	1242	1904	2915
	b	3632	11798	3845	7061	6227	737	1456	2088	2725
T2	a	750	898	1244	1246	1210	1400	1380	1424	1634
	b	792	920	1244	1450	1378	1482	1690	1498	1530
T3	a	155440	8986	4062	3160	21180	-	11661	2284	43564
	b	163060	10798	4557	2731	20544	-	12554	2425	42317
T4	a	16280	22359	133150	22228	6782	6471	1152	4575	1100
	b	19030	20449	139510	25202	6632	6637	1082	4597	1415
T5	a	1284	1549	3572	2956	6869	5714	1609	1338	1713
	b	1253	1715	3745	1613	7136	5865	1310	1345	1752
T6	a	2027	22909	2796	1585	1209	847	592	368	554
	b	1960	23462	2988	1256	1145	895	590	365	565