FINAL REPORT

Insecticide Resistance in *Helicoverpa* spp.

DAN 33L

Summary

The project DAN 33L involved studies of insecticide resistance in the major cotton pests *Helicoverpa armigera* and *Helicoverpa punctigera.* The aims of the project were : to investigate the mechanisms of pyrethroid insecticide resistance in *H.armigera*; to develop *Helicoverpa* bioassay techniques against ovicides, stomach poisons and first instar larvae; to monitor *H.armigera* resistance to endosulfan, carbamates and organophosphates and to monitor the response of *H.punctigera* to insecticides. The findings of these studies are summarised below.

In 1983, at the onset of pyrethroid resistance in Australian *H.armigera,* three resistance mechanisms were identified. They were : a strong nerve insensitivity (*Super - Kdr*) , penetration resistance (*Pen*), and a factor which was overcome by piperonyl butoxide (*Pbo*). Nerve insensitivity was the major cause of pyrethroid resistance and conferred high order resistance ~100 times. From 1987 to 1990, to monitor accurately the effectivness of the Australian *Helicoverpa* insecticide resistance management strategy, we conducted a survey of resistance mechanism frequencies in field collected resistant *H. armigera.* The relative importance of the *Pen* and *Pbo* mechanisms in resistant *H. armigera* have increased, as *Kdr* has decreased in gene frequency and potency. *Pen* and *Pbo* confer only low order resistance. The impact of the *Helicoverpa* insecticide resistance management strategy on pyrethroid resistance in *H.armigera* is discussed.

A method for the bioassay of contact insecticides against larval first instar *Helicoverpa* spp. is described. "Brown eggs" were sprayed with formulated larvicides, just prior to hatch. The neonate larvae received insecticide dosage from the exterior of the shell and sprayed surrounds. Trials pyrethroids and organophosphorous insecticdes, showed a close relationship between the concentration of active ingredients and mortality in first instar larvae. The method sucessfully distinguished between pyrethroid resistant and susceptible *H. armigera.* The results were compared with bioassay of third instar larvae by topical application. The importance and advantages to the Australian *Helicoverpa* insecticide resistance management strategy of resistance monitoring in first instar larvae are discussed.

H. armigera larvae were collected from NSW and Queensland from 1983 to 1990 and bioassayed with methomyl and thiodicarb. Methomyl was tested by topical application on 3rd instar larvae. New bioassay techniques for thiodicarb, a stomach poison and methomyl as an ovicide were developed and are described. Baseline susceptibility data for thiodicarb are presented, as is evidence of widespread resistance to methomyl in *H. armigera* larvae. Resistance to methomyl in a selected strain was estimated as approximately 23 fold, while in field strains, resistance did not exceed 11 fold. Resistance was not expressed by the egg stage. These data are discussed with reference to possible resistance mechanisms and the Australian *Helicoverpa* resistance management strategy.

Organophosphate were bioassayed against Australian *Helicoverpa armigera* collected from NSW and Queensland. Methyl parathion, sulprofos and profenofos were tested against 3rd instar larvae. Baseline susceptibility data for methyl parathion are presented, as well as evidence for incipient organophosphorous resistance. These data are discussed with reference to the Australian *Helicoverpa* resistance management strategy.

H. armigera larvae were collected from New South Wales and Queensland from 1974 to 1990 and laboratory cultures were established. Endosulfan was topically applied to 3rd instar larvae of the F1 generation of these strains, and a susceptible reference strain. The highest levels of endosulfan resistance (>50-fold) were recorded in 1974. Resistance was barely detectable from 1977 to 1983 but since then resistance has become widespread. However, resistance levels have remained generally low with only 2 of the 106 strains tested showing levels of resistance above 10-fold. The highest level of resistance recorded after 1975 was 23 fold and laboratory selection with endosulfan increased resistance in this strain to 163-fold. These data are discussed in terms of cyclodiene use on cotton and the resistance management strategy *H. armigera* which was implemented in NSW and Queensland during 1983.

Helicoverpa punctigera were collected from field locations in Australia, principally from New South Wales and Queensland, between 1974 and 1989. *H.punctigera* were bioassayed with deltamethrin, fenvalerate, DDT, endosulfan, carbaryl, methomyl and methyl parathion. Bioassay was by topical application on third instar larvae. Baseline susceptibility data are presented for DDT, endosulfan, carbaryl, methomyl and methyl parathion. There was evidence of heterogeneity of response to pyrethroids, in particular to deltamethrin. These data are discussed with reference to the ecology of *Helicoverpa* spp and the Australian *Helicoverpa* resistance management strategy.

COTTON RESEARCH AND DEVELOPMENT CORPORATION

Final Report - Project DAN 33L

INSECTICIDE RESISTANCE IN *HELICOVERPA* SPP

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FINAL REPORT

Insecticide Resistance in *Helicoverpa* spp.

DAN 33L

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Introduction

A complex of pests attacks the Australian cotton crop. None are more important than the cotton bollworm, *Helicoverpa armigera* (Hubner) and the native budworm *Helicoverpa puctigera.* Insecticides are considered essential for their control. *H. armigera* has a long history of insecticide resistance in Australia - to DDT in the early 1970's and more recently, in 1983, to the synthetic pyrethroids . *H.punctigera* has not developed field resistance to any chemical. Since 1983, *Helicoverpa* insecticide resistance has been the subject of an insecticide resistance management program in NSW and Queensland which has restricted the use of pyrethroids and other insecticides in cotton and other crops.

The aims of this project were:

1. To study the mechanisms of insecticide resistance in *H.armigera.*

2. To develop bioassay techniques against 1st instar *Helicoverpa* larvae, ovicides, stomach poisons and other insecticides with novel modes of action or unusual modes of entry.

3. To monitor *H.armigera* resistance to endosulfan, the carbamates and organophosphates.

4. To monitor the response of *H.punctigera* to insecticides.

Pyrethroid resistance mechanisms in *H.armigera*

Introduction

Resistance to pyrethroid insecticides is common in insects and mechanisms are varied. The pyrethroid resistance mechanisms fall into three types; reduced nerve sensitivity to pyrethroid poisoning, reduced pyrethroid penetration through the cuticle and enhanced pyrethroid metabolism. Resistance has been found usually to be due to a combination of these factors. This report details investigations into the mechanisms of pyrethroid resistance in Australian *H.armigera*.

Methods and materials

Susceptible and pyrethroid resistant strains of *H. armigera* were used in this study. The initial susceptible strain was collected from Bellingen, NSW in 1983. Strains were obtained from field collections in Queensland and NSW from 1983-1990.

Insecticides and bioassay

Technical grade fenvalerate (99% a.i.), permethrin (98%.a.i. 40:60 cis trans ratio), cypermethrin (98% a.i.. 60:40 cis trans ratio) and deltamethrin (97% a.i.) were used for bioassay. Serial dilutions were prepared with acetone. Bioassay methods for topical application, have been described previously (Gunning, et al. 1984). Piperonyl butoxide (99% a.i.), S,S,S - tributyl phosphorotrithioate (DEF) (95% a.i.) and profenofos (99% a.i.) were used as synergists diluted in actone and topically applied to *H. armigera* larvae 30 min. before the application of pyrethroids. Synergists and pyrethroids were applied at a ratio of 100:1, (except for profenofos, because of its intrinsic, toxicity which was applied at a 1:1 ratio). Fenvalerate was injected into 30-40 mg larvae using a 1 µl syringe. Solvent injections were not harmful and did not cause significant haemolymph loss. Dosage mortality data were analysed by probit analysis.

Electrophysiological studies

Fifth instar larvae, weighing 350-400 mg were used for these studies. The larvae were pinned to a plasticine coated dish. The larvae were envicerated by dorsal dissection and the ventral body wall muscles were flooded with saline. Muscles in the body wall were used to make measurements of spontaneous activity from the peripheral nerves. Peripheral nerves were picked up by a suction electrode and the preparation grounded using a stainless steel insect pin. The recording electrode was connected to pre amplifier. The input was displayed on a cathode ray oscilloscope and the signal was fed into a window discriminator nerve action potentials were counted by an Xcount Apple progammable timer board and an Apple IIE computer

The experimental procedure was initially to record spontaneous nerve activity in saline and then to replace it with saline containing various concentrations of pyrethroid. 10^{-5} M fenvalerate was the minimum concentration to induce repetitive firing in the susceptible strain, within 5 mins, and was chosen as the diagnostic concentration (Fig.1). The number of action potentials in each minute were counted. Experiments were conducted at 25 ± 1°C.

Penetration and distribution studies

^{14}C O-S- fenvalerate (18.4 mCi / m mole) and [^{14}C-cyclopropyl] cypermethrin (50 mCi / m mole) were used for this work, aliquots (1µl) in acetone solution were applied topically to the dorsum of 325-375 mg 5th instar larvae, using a microsyringe. There were 15 insects in each treatment group. The larvae were kept in 28 ml plastic contaimers without food, for periods up to 24 h after dosing. The resistant and susceptible strains were dosed with 0.25 µg pyrethroid / larva. This dose corresponded to the approximate LD_5 of fenvalerate and cypermethrin in the resistant strain. Experiments were conducted at 25±1°C.

At appropriate times after dosing, groups of larvae were dissected. Each larva was divided into the head, integument, excreta, fat body and gut. The haemolymph was collected by bleeding from a pro-leg and the excreta in the rearing container was reserved. The insect fractions were combusted with a Packard Model 306B Tricarb sample oxidiser with Carbsorb as the ^{14}C trapping agent and radioassayed with Permafluor V with a Packard TRICARB Scintillation Counter. The counting efficiency was established by an external standard and all the results were calculated as disintergrations per minute.

As the dissection of *H. armigera* was especially time consuming and thus unsuitable for the screening of large numbers of insects for penetration resistance, we devised a more rapid method to investigate insecticide penetration. Intact larvae were rinsed with 5x1ml aliquots of acetone and the acetone washes for each larva pooled, evaporated and radioassayed with Permafluor V. Insecticide pentration data generated by combustion of the integument, or by cuticular wash were well correlated (Table 1, Fig 2).

Field survey of the frequency of pyrethroid resistance mechanisms in NSW and Queensland 1987-1990.

Pyrethroid resistance frequency in *H. armigera* has been monitored, via a discriminating dose technique, to test the effectivness of the *Helicoverpa* resistance management strategy. Following concern in 1987 about increasing *H. armigera* pyrethroid resistance frequency in NSW and Queensland, a survey of pyrethroid resistance mechanisms in resistant field *H. armigera* was undertaken. *H. armigera* eggs and small larvae were collected throughout the summer cropping season (from unsprayed NSW and sprayed populations from Namoi / Gwydir and Emerald) and bioassayed with a discriminating dose of fenvalerate. Survivors were tested, for pyrethroid penetration resistance (*Pen*) and nerve insensitivity (*Kdr*). Weekly *Pen* and *Kdr* frequencies were counted and percentages calculated for Stages 1, 2 and 3 of the *Helicoverpa* resistance management season. More than 50 *H. armigera* per stage from each area were assayed for *Pen* and *Kdr*. The frequency of the *Pbo* factor in unsprayed NSW populations was determined, by pre-treating samples (n=30) from each *H. armigera* culture with Pbo prior to application of the fenvalerate discriminating dose. Mortality, caused by the fenvalerate / Pbo mix compared to fenvalerate alone was assessed.

Results

Bioassay

The fenvalerate selected Emerald strain was 60-100 times resistant to fenvalerate, cypermethrin, and deltamethrin, compared with the Bellingen susceptible strain (Table 1).

Electrophysiology, 1983

Spontaneous nerve activity was measured using the body wall tissue of susceptible and resistant larvae (n=50). Baseline nerve activity varied from larva to larva, ranging from 5-50 discharges per min., with no differences between resistant and susceptible strains. Nerve response to fenvalerate was the repetitive burst discharge, characteristic of both Type I and Type II pyrethroids.. Treatment of the susceptibles rapidly caused multiple nerve firing and long trains of spikes that lasted many milliseconds. At the diagnostic concentration of 10^{-5} M fenvalerate, there were clear differences between the susceptible and resistant strains in the interval between insecticide treatment and the onset of multiple nerve firing (Table 2). 10^{-5} M cypermethrin, deltamethrin and permethrin produced similar results. Smaller numbers (n= 20) of discriminating dose survivors, from other resistant sites (Moree, Narrabri, Kinagaroy) were assayed with fenvalerate and were similarly found to be less sensitive to the effects of insecticide than the susceptible strain. Nerve insensitivity was thus correlated to the distribution of pyrethroid resistance in the field. One hundred percent of field resistant *H. armigera* assayed showed nerve insensitivity to pyrethroids.

We investigated the inheritance of *Kdr* by crossing the selected Emerald resistant strain with the susceptible strain. Thirty of the F_1 progeny were assayed for nerve response to fenvalerate. The time to initiation of burst discharge of the F_1 (Table 2) was clearly intermediate between the susceptible and resistant parental strains. The nerve insensitivity factor appeared to be inherited as an incompletely dominant factor.

Penetration and distribution studies , 1983

Results of experiments to study the penetration and distribution of ^{14}C fenvalerate and cypermethrin in the selected Emerald pyrethroid resistant and susceptible strains over time are shown (Table 3). There were no significant differences in the amount of pyrethroid recovered from the fat or haemolymph. Penetration of pyrethroids through the cuticle was more rapid in the susceptible larvae than the resistants. After four hours only 25% of applied ^{14}C cypermethrin remained on the cuticle compared to 55% on resistants. Fenvalerate pentration rate was similar. The passage of ^{14}C pyrethroids from the gut to the excreta was also more rapid in susceptibles than resistants . We studied the inheritance of reduced pyrethroid penetration through the cuticle by mating the resistant (selected) and susceptible strains and treating the F$_1$ progeny with ^{14}C cypermethrin. There were similarities between the rates of pentration and elimination between the F$_1$ (RxS) and the resistant parent strain, (Table 3). These data suggest that the factors controlling penetration may be dominant .

The dissection of *H. armigera* was too time consuming for screening large numbers of insects so we devised a more rapid procedure to investigate insecticide penetration. It utilised an acetone rinse (5x1 ml) to remove any ^{14}C fenvalerate adhering to the larval cuticle and allowing it to evaporate, before the addition of scintillant . Recovered ^{14}C versus time, in resistant and susceptible strains, is plotted in Fig. 2. The data are very similar to those obtained from the cuticle by sample oxidation (Table 3). We later used the cuticle wash technique to screen large numbers of field collected *H. armigera* for pyrethroid penetration resistance for a resistance mechanism survey in 1987 - 1990.

Synergism studies in 1983.

Synergist action may infer insecticide metabolism in resistant insects. The effects of several pyrethroid synergists (piperonyl butoxide, DEF and profenofos) were investigated against the Emerald resistant selected *H. armigera* by pre-treatment with the synergist and prior to the fenvalerate discriminating dose. The discriminating dose of fenvalerate alone, or with DEF and profenofos, did not kill resistants. However the addition of piperonyl butoxide (Pbo) increased mortality to 15%, (Table 4). We found the synergists did not increase the efficacy of fenvalerate against susceptible *H. armigera.*

Relative importance of resistance mechanisms in 1983

The relative contributions to pyrethroid resistance of *Kdr* , *Pen* and the *Pbo* synergism factor were studied by injecting fenvalerate directly into the insect. Resistant *H. armigera* larvae (30-40 mg), were injected with the fenvalerate discriminating dose with or without 10 µg of Pbo (by microsyringe, in 1µl of acetone). Fenvalerate injected alone did not kill the larvae, whilst fenvalerate injected with Pbo caused only slight mortality (10 ± 5 %). The surviving larvae were retained and assayed for *Kdr* at the fifth instar . Each of 30 larvae showed nerve insensitivity to pyrethroids , the mean time to initiation of repetitive discharge was 20.1 ± 2.2 min (compared to 4.3 ± 1.7 for susceptibles, Table 2). Nerve insensitivity thus seemed to be the primary cause of pyrethroid resistance.

Field survey of the frequency of pyrethroid resistance mechanisms in NSW and Queensland , 1987-90

Nerve insensitivity

We surveyed for the field frequencies of nerve insensitivity , in sprayed and unsprayed *H. armigera* , from 1987 to 1990, (Fig 3). In 1983 high order nerve insensitivity was primarily responsible for pyrethroid resistance in *H. armigera.*, but the 1987-90 survey showed some considerable changes in its potency. The mean time to repetitive discharge in *Kdr H. armigera* was 12 + 1 mins, considerably less than that of the 1983 resistants. This factor, when genetically isolated, only delayed the onset of nerve poisoning and conferred no actual fenvalerate resistance (R.V. Gunning and J. Daly).

In 1987/88, in all areas and throughout the season, 50-70 % of resistants had this low grade *Kdr*, but in 1988/89, the frequency fell (in all populations), to approximately 10 % by late season. In 1989/ 90, there was a scarcity of *H. armigera* on cotton. However resistants from unsprayed populations were available for assay throughout the year and their data suggested that *Kdr* frequency had slightly increased, to exceed the levels of the previous season. The source of the increased numbers of *Kdr H. armigera* appeared to be the Namoi Valley. The early season, higher frequency of *Kdr* in the Namoi /Gwydir insecticide sprayed *H. armigera*, was not found in unsprayed *H. armigera* until late season. These data were correlated with a a very low summer density of *H. armigera* in the Namoi / Gwydir cotton and little evidence of early season *Helicoverpa* migration .

Penetration resistance frequency

The frequency of *Pen* was unknown in 1983 and this mechanism was demonstrated to be a very a minor contributor to resistance. Yet, the 1988/90 survey of its frequency showed it to be common (Fig 4). *Pen* frequency ranged from 58 to 82 % in 88/89 season, with little significant variation between sprayed and unsprayed insects, or the time of year. In 1989/90 Stage 1 and Stage 2 *Pen* frequencies were also high, from 83 - 95% , but in late season *Pen*, in the NSW refugia had declined to 50%.

Piperonyl butoxide synergism factor frequency

The frequency of resistant *H. armigera* from the NSW refugia carrying the *Pbo* factor was monitored during 1988 - 90 (Fig 5). The frequency increased from 50 to 80% in 1988/89 but had declined to less than 60% in 1989/90.

Discussion

1983 Resistance mechanisms

In 1983, at the onset of pyrethroid resistance, we found there were three mechanisms of pyrethroid resistance in *H. armigera* , namely reduced pyrethroid penetration , a nervous system that was less sensitive to pyrethroid attack and had another factor which was synergisable by Pbo. Nerve insensitivity was the most important mechanism. *Pen* and the *Pbo* factor, were minor mechanisms, possibly important as modifiers of the *Kdr* resistance gene.

Nerve insensitivity

Compared to the susceptible strain, there was clear evidence of widespread high order nerve insensitivity in resistant *H. armigera* , indicated by the very delayed, or absence pyrethroid induced repetitive discharge (Table 2). It was present in all resistant *H. armigera* from NSW and Queensland. This strong nerve insensitivity, which was correlated with strong pyrethroid resistance, is suggestive of the *Super Kdr* allele. *Super- Kdr* insects have *Kdr* like mechanisms that are correlated with strong pyrethroid resistance . Our findings are consistent with those of S. N. Irving , (ICI Jealots Hill Research Station, U.K.), who also studied Australian <u>H. armigera</u> in 1983. Recording miniature post synaptic potentials (mEPSPs) from the intersegmental muscles in the body wall of larvae, Irving was able to show that cypermethrin and fenvalerate were less effective at causing an increase in mEPSP rate leading to synaptic block. Pyrethroid nerve responses in resistant strains were 100-500 times less than for susceptibles. It was suggested that these levels of nerve insensitivity were due to a *Super- Kdr* resistance factor.

Our studies of *H. armigera Super-Kdr* inheritance suggested the factor was incompletely dominant (Table 2). The nerve response times of the F_1 were clearly intermediate between the susceptible and resistant parents. This hypothesis is supported by findings that pyrethroid resistance in *H.armigera* was incompletely dominant and that in 1983 nerve insensitivity was the major cause of pyrethroid resistance. Genetic studies of the *Kdr* mechanism in other insects have shown that a single homozygous recessive gene was responsible.

4.1. 2. Penetration resistance

In 1983, the cuticle of resistant *H. armigera* larvae resisted the entry of fenvalerate and cypermethrin (Table 3). After four hours, only half as much pyrethroid had passed through the cuticle of the resistants compared to the susceptible strain. Pyrethroids also passed, via the fat body and haemolymph, to the gut and excreta much more rapidly in susceptibles. The more rapid excretion of insecticides by susceptibles may show that the C^{14} insecticides accumulated in the gut of resistants were non-toxic, but may also reflect the slower resistant cuticle penetration and reduced insecticide availablity.

H. armigera pyrethroid penetration resistance appeared dominant because the progeny of resistant x susceptible crosses were similar to the resistant parent.

Pbo synergism

Our 1983 data showed that piperonyl butoxide was a minor synergist of pyrethroids in the resistant strains, increasing resistant kill by approximately 15% (Table 4). Little is known concerning the mode of action of synergists, but piperonyl butoxide is known to inhibit microsomal mono - oxygenases. While synergism studies that imply biochemical mechanisms of resistance should be interpreted very cautiously , Pbo / pyrethroid synergism in *H. armigera* may suggest a role of the mono - oxygenase system. Alternatively, Pbo can also facilitate insecticide penetration through the cuticle.

Field survey of resistance mechanisms 1987-90

Following the 1983 diagnosis of pyrethroid resistant *H.armigera* (Gunning et al, 1984) and the identification of three resistance mechanisms, a *Helicoverpa* resistance management strategy was instituted. The strategy has restricted pyrethroid use on all crops in inland northern NSW and Queensland to approximately one of the 4-5 generations of *H. armigera* that can occur each year. Our data show that there have been considerable changes in the resistance mechanism frequencies in *H. armigera* since the onset of the resistance management strategy.

Since 1983 the nerve insensitivity mechanism has changed in two ways. Its population frequency and potency have both declined, (Fig 3). In 1983 a *Super -Kdr* like mechanism was the major cause of resistance. But in 1990 there was only a low order *Kdr* factor which delayed nerve poisoning. The partial withdrawl of pyrethroid insecticides from use against *H. armigera* since 1983 has lessened the selection pressure on the *Super-Kdr* resistance mechanism, probably causing a gradual decline in its frequency. There are two possible explanations for the loss of potency of the mechanism. Reduced selection pressure may have caused a decline in resistance gene expression Alternately, while *Super Kdr* was lost, a lower order pyrethroid nerve insensitivity gene may have been selected, or perhaps have already been present, masked by *Super Kdr.* The selection of additional resistance mechanisms, which may supplant the original resistance characteristics of the population is known. Differing *Kdr* alleles are also known. *Super-Kdr* and *Kdr* are well documented. Another nerve mutation (*Nap*) has been described for *Drosophila melanogaster*. *Nap* reduced nerve sodium channel density, and was correlated with pyrethroid and DDT resistance. The magnitude of resistance conferred by *Nap* was much smaller than that found in *Kdr* and *Super-Kdr*. Low order nerve insensitivity found in <u>H. armigera</u> may be similar to *Nap.*

The *Pen* and *Pbo* factors had high frequencies during 1988 -90, but there was some evidence of a decline in 1989/90 . In the absence of a strong nerve insensitivity factor, *pen* and *Pbo* are responsible for the present high frequency, low order resistance (~x20 for fenvalerate) in NSW and Queensland.

Conclusions

Our studies show the impact of the *H. armigera* insecticide resistance management strategy on pyrethroid resistance. The importance of *Super-Kdr* as a resistance mechanism has decreased with a concomitant increase in the relative importance of the *Pen* and *Pbo* factors. These changes have coincided with an increasing resistance frequency, despite decreased pyrethroid use. The reasons for the selection of the *Pen* and *Pbo* resistance mechanisms are not clear. Cross resistance selection of *Pen* and *Pbo* by other insecticides is improbable, as other insecticides used against *H. armigera* (endosulfan, methomyl, thiodicarb, sulprofos, profenofos and methyl parathion) have no pyrethroid cross resistance.

It is posible that the removal of pyrethroid selection pressure has caused the loss of the *Super-Kdr* mechanism . *Super-Kdr* is normally an intractable mechanism that confers such high order resistance that resistants are difficult to control. *Pen* and *Pbo* confer only low order pyrethroid resistance, this is less of a challenge to pyrethroid efficacy.

Resistance to pyrethroids is complex and clearly is sensitive to pyrethroid resistance management decisions. While the adjustment of pyrethroid selection pressure has ameliorated the danger of *Super Kdr*, any increase in rates would undoubtedly exacerbate the situation. The overuse of pyrethroids, synergised by Pbo, in the field should be avoided for similar reasons.

Figure 1.

Time to pyrethroid (fenvalerate) induced burst discharge recorded from the body wall nerves of susceptible H. armigera larvae. Vertical bars represent standard errors, N = 20 larvae per fenvalerate concentration.

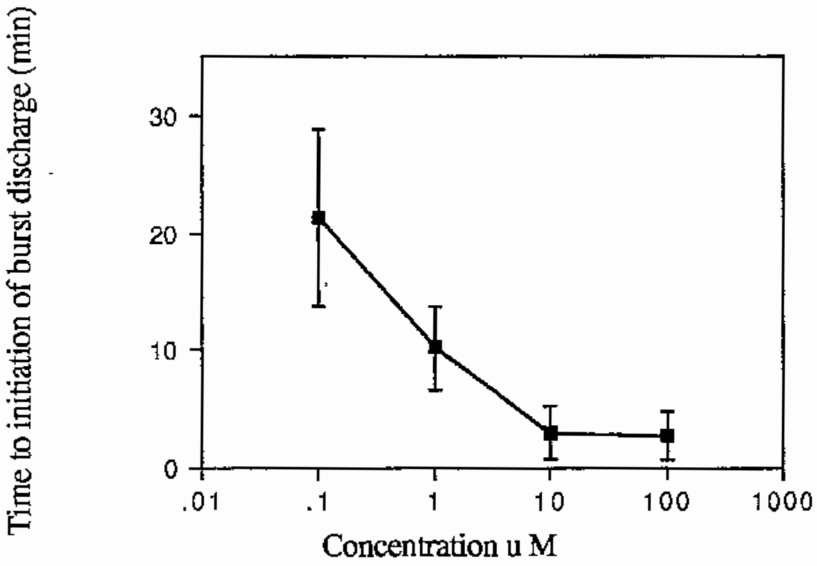


Figure 2.

Penetration of ^{14}C labelled fenvalerate through the cuticle of pyrethroid resistant (—□—) and susceptible (—■—) H. armigera larvae, measured from an external cuticle wash. Vertical bars represent standard errors, N = 20 larvae per sampling time.

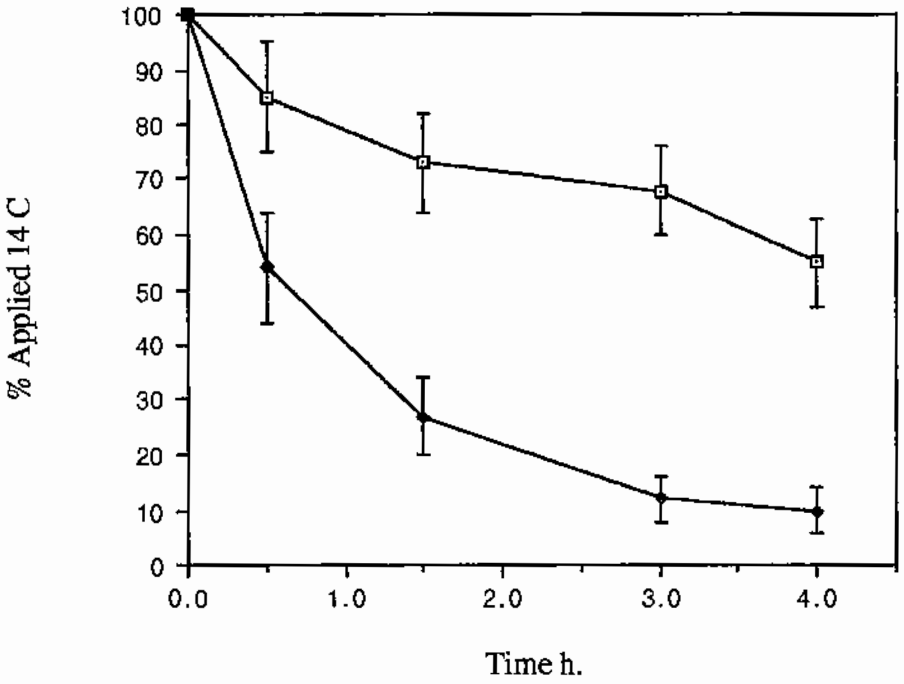


Figure 3

Frequency of nerve insensitivity in pyrethroid resistant H. armigera, 1987 - 1990. The larvae were from unsprayed NSW areas (refugia) and sprayed cotton crops of NSW and Queensland (Namoi / Gwydir and Emerald). Vertical bars represent standard errors. More than 50 larvae per stage from each area were assayed for *Kdr*. NSW Refugia ■ , Namoi ▒ , Emerald ▨ .

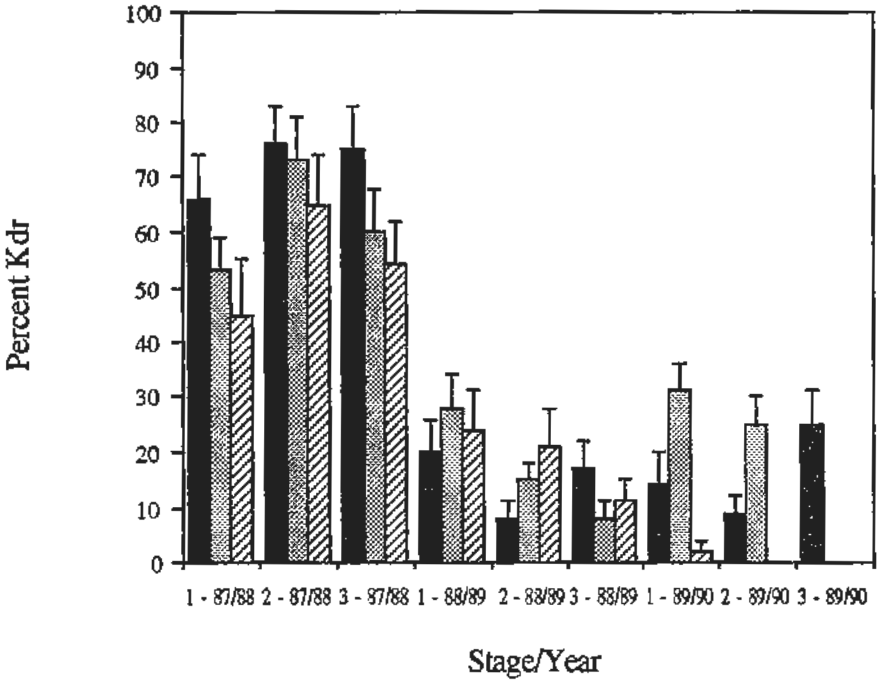


Figure 4
Frequency of the *Pen* resistance mechanism in pyrethroid resistant H. armigera, 1988 - 1990. The larvae were from unsprayed NSW areas (refugia) and sprayed cotton crops of NSW and Queensland (Namoi / Gwydir and Emerald). Vertical bars represent standard errors. More than 50 larvae per stage from each area were assayed for *Pen*. NSW Refugia ■ , Namoi ▩ , Emerald ▨

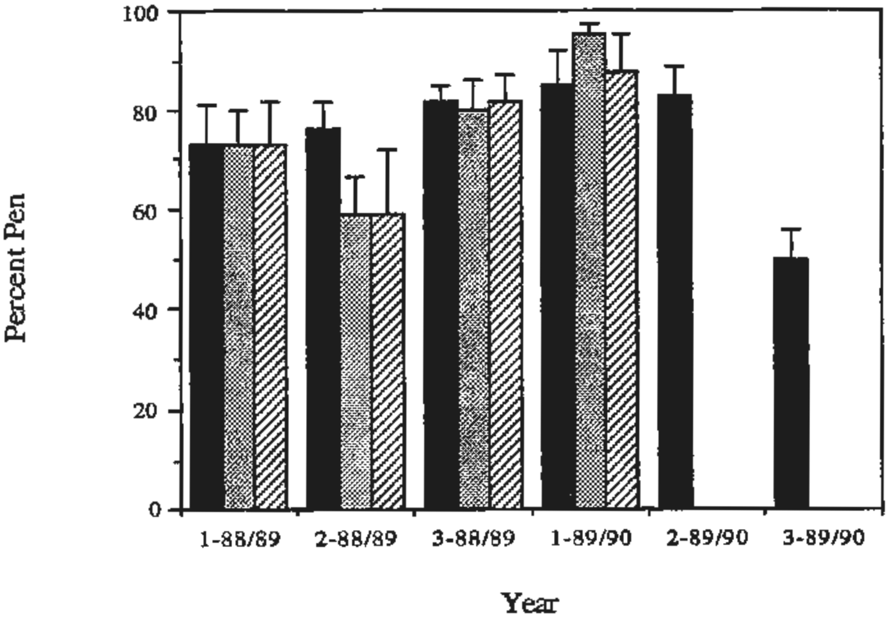


Figure 5
The frequency of the *Pbo* synergism resistance mechanism in pyrethroid resistant H. armigera larvae collected from unsprayed NSW refugia 1988 - 89. Vertical bars represent standard errors. Thirty larvae from each of 29 (88/89) and 30 (89/90) field collected cultures were pre - treated with Pbo prior to the application of the discriminating dose of fenvalerate. Stage 1 ■ , Stage 2 ▩, Stage 3 ▨ .

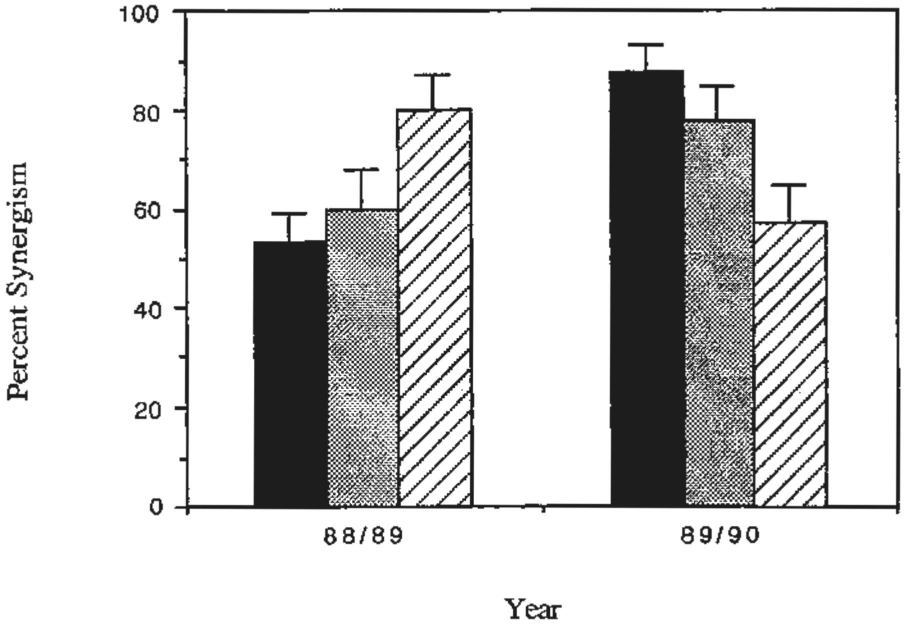


Table 1 Toxicity of topically applied insecticides to Helicoverpa armigera larvae.

Insecticide	Strain	Year	LD_{50} µg/larva	Slope ± S.E.	R.F#
fenvalerate	Bellingen	1983	0.03	3.6 ± 0.2	-
fenvalerate	Lab Strain	1989	0.03	2.6 ± 0.1	-
fenvalerate	Emerald(sel.)*	1983	3.1	2.9 ±0.2	103
cypermethrin	Bellingen	1983	0.03	4.3 ± 0.3	-
cypermethrin	Emerald(sel.)	1983	2.5	2.8 ± 0.2	83
deltamethrin	Bellingen	1983	0.01	2.6 ± 0.2	-
deltamethrin	Emerald(sel.)	1983	0.6	2.4 ±0.2	60

* Resistant Emerald strain selected from survivors of greater than 10 times the discrininating dose of fenvalerate.

Resistance factors were calculated as the ratio of LD_{50} Emerald (sel) / LD_{50} Bellingen susceptible strain.

Table 2

Time to induction of repetitive nerve firing by fenvalerate in 1983 pyrethroid resistant (Emerald, selected) and susceptible (Bellingen) H. armigera larvae (350 - 400 mg) and their F_1 progeny.

Strain	Time (mins.)#
Susceptible	4.3± 1.7
Resistant *	22.0± 4.4
Resistant xSusceptible F_1	16.1 ± 0.8

* fenvalerate did not induce repetitive nerve firing at all, in 33% of resistants assayed.
# Each value is the mean (± S.E.) of four replicates of 10 individuals

Table 3. Penetration and distribution of ^{14}C pyrethroids in body tissues of susceptible (Bellingen) and resistant (Emerald selected) H. armigera. and their F$_1$ progeny in 1983, analysed by sample oxidation. Data are means of 3 replicates of 5 insects (± S.E.).

	cypermethrin % applied ^{14}C			fenvalerate % applied ^{14}C		
Time h.	0.5	1.5	4.0	0.5	1.5	4.0
cuticle -S	70 ± 7	40 ± 5	25 ± 4	68 ± 5	40 ± 5	22 ± 4
cuticle -R	74 ± 6	70 ± 6	55 ± 5	70 ± 6	70 ± 9	60 ± 6
cuticle- SxR	75 ± 7	64 ± 6	60 ± 6			
fat -S	10 ± 3	7 ± 4	7 ± 3	8 ± 3	8 ± 4	12 ± 5
fat- R	8 ± 4	12 ± 5	10 ± 3	6 ± 2	5 ± 3	8 ± 3
fat- SxR	12 ± 5	16 ± 5	20 ± 5			
haemolymph -S	10 ± 4	12 ± 4	5 ± 2	2 ± 1	6 ± 2	20 ± 5
haemolymph -R	0	2 ± 1	5 ± 2	1	3 ± 1	5 ± 2
haemolymph - SxR	1	1	2 ± 1			
gut - S	9 ± 3	28 ± 5	10 ± 3	22 ± 5	18 ± 4	10 ± 2
gut - R	10 ± 3	18 ± 4	35 ± 6	18 ± 4	25 ± 6	25 ± 5
gut - SxR	6 ± 2	20 ± 5	10 ± 3			
excreta -S	0	30 ± 5	42 ± 8	1	5 ± 2	20 ± 4
excreta - R	0	3 ± 1	12 ± 3	0	0	2 ± 1
excreta - SxR	1	0	4 ± 1			
head - S	0	2 ± 1	1	0	1	2 ± 1
head - R	0	0	1	0	1	1
head - SxR	1	1	0			

Table 4

Toxicity in 1983 of fenvalerate* / synergist mixtures after topical application to pyrethroid selected H. armigera from Emerald.

Insecticide /Synergist	% Mortality
fenvalerate	0
fenvalerate + 10 µg piperonyl butoxide	15
fenvalerate + 10 µg DEF	0
fenvalerate + 0.1 µg profenofos	0

* Discriminating dose of fenvalerate 0.12 µg per 30-40 mg larvae.

Bioassay Techniques for Contact Insecticides Against 1st Instar *Helicoverpa* Spp.

Introduction

The insecticide resistance management strategy on *H. armigera* resistance gene frequencies has been monitored by insecticide bioassay since 1984. Bioassay has been by topical application of contact insecticides, such as fenvalerate, to 3rd instar larvae. These methods are similar to those recomended by the Entomological Society of America.

During the resistance studies, it has become evident that there are a number of drawbacks with the 3rd instar bioassay method. It is necessary to rear large numbers of *Helicoverpa* larvae to 3rd instar, this is both expensive and time consuming. Canibalism necessitates that each larva be reared to 3rd instar individually. The results are not available for many days, or even weeks after field collection of the insects.

To eliminate some of the problems, thus to facilitate *Helicoverpa* resistance monitoring, method has been devised for the bioassay of contact insecticides against neonate 1st instar larvae. The method is effectivne in discriminating between *H. armigera* and *H. punctigera* and pyrethroid resistant and susceptible *H. armigera*

Materials and Methods

Susceptible and pyrethroid resistant strains of *H. armigera* were used in this study. The susceptible strain was collected in 1983, from Narrabri NSW. The resistant strains were collected from Narrabri and Emerald, Q. in 1983. Resistant selected strains were bred from the Enerald culture. The *H. punctigera* used in this study were collected from Narrabri in1983.

Formulated E. C 's of fenvalerate (Sumicidin^R, Shell)) were diluted in distilled water to form stock solutions. Test concentrations were prepared by serial dilution with distilled water. Fresh solutions were prepared daily. *H. armigera* and *H. punctigera.* eggs were collected on papr as laid and retained at 25 °C and 90% humidity. The eggs were left to develop to the brown egg stage . Two hundred eggs were tranferred by paintbrush to filter paper discs (10 cm diameter). The discs were then sprayed with insecticide solutions using a Potter Tower. The Potter Tower produced an even spray deposit of 1.5 mg / cm^2. Control eggs were sprayed with distilled water. Three replicates of 200 eggs were sprayed with each insecticide concentration. The papers were allowed to dry, then transfered to an incubator at 25 °C and 90% humidity. The papers were spaced as to prevent the exchange of larvae between filter papers with extraneous larvae. A white fluorescent light (20 W) to the rear, provided constant illumination. Hatching occurred within 24 h and the neonate larvae were immediately attracted away to the light source, unless affected and killed by insecticide exposure. The filter papers were examined under a magnifing light and the numbers of dead eggs and larvae were recorded. None of the insecticides tested, had any noticeable ovicidal effect. First instar larval mortality was recorded as the percentage of dead larvae remaning on the treated filter papers. Larvae that had sucessfully left the filter papers were assessed as 'alive'. The results were corrected for control mortality and the data were analysed by Probit analysis .

Results

Results obtained for the fenvalerate bioassay of *H. punctigera* and *H. armigera* (resistant and susceptible) strains are shown in Table 5. First instar bioassays were as readily able to distinguish between the two *Helicoverpa* species and between pyrethroid resistant strains of *H. armigera*. as the standard 3rd instar topical application method.

The concentation / mortality curves for first instar *H. punctigera* and the pyrethroid susceptible *H. armigera* strain both had slope values of 3.0, indicating little heterogeneity of response. *H. armigera* were intrinsically more tolerant (2 times), to fenvalerate than *H. punctigera* .The pyrethroid resistant field strain showed increased LC_{50} value and a low slope value indicating heterogeneity in response to fenvalerate. The fenvalerate selected *H. armigera* strains showed slope values increasing in proportion to the severity of selection These data are very similar to that produced by the 3rd instar bioassay (Table 5.). The pyrethroid resistance factors, calculated for first instar larvae were little lower than those obtained by third instar bioassay.

Discussion

The data has shown that contact insecticides, such as the pyrethroids, may be accurately bioassayed on neonate 1st instar *Helicoverpa* larvae. Our method, which involved contact of the first instar larvae with a insecticide sprayed surface, yielded resistance information, comparable with the standard 3rd instar topical application methods . The 1st instar bioassay distinguished well between *H. armigera* and *H. punctigera* , by susceptibility to pyrethroid. The assay also differentiated between fenvalerate resistant and susceptible strains of *H. armigera* .

The pyrethroid resistance factors, calculated for first instar larvae were lower than those obtained by third instar bioassay. However, since at least 5 - 10 times more *Helicoverpa* individuals were used to generate the first instar dosage mortality data their results are undoubtedly the more reliable.

The 1st instar bioassay method for pyrethroid insecticides against *Helicoverpa* spp. has several advantages over the standard 3rd instar bioassay method. Firstly we were able to test many many more individuals thus increasing statistical reliability. Secondly, using large numbers greatly increases the chances of detecting resistance at low frequencies,the basic requirement any resistance monitoring program. Lastly 1st instar bioassay is fast and inexpensive, eliminating much of the expensive and labour intensive *Helicoverpa* rearing.

The 1st instar bioassay proceedure, used as a discriminating dose technique will be a valuable aid to the management of *Helicoverpa* pyrethroid resistance in Australia. The insecticide resistance management strategy requires reliable methods to aid in the monitoring for and the documentation of resistance .If resistance monitoring programs are to be of any practical use to resistance management, they need to detect resistant individuals at very low resistance frequencies. The sample sizes of insects required to detect low frequencies of resistant individuals is necessarily large. The first instar pyrethroid bioassay technique described here was able to use very large numbers of *Helicoverpa* . The techniques reported here should provide fast inexpensive and reliable methods needed for pyrethroid insecticide resistance monitoring of *H. armigera* in Australia.

Table 5

Toxicity of fenvalerate to 1st and 3rd instar *H. punctigera* and *H. armigera* larvae.

First Instar

Species	Site	Date	Number	LC 50	Slope	Fiducial Limits	RF
H. punctigera	Narrabri	1/83	1361	0.0024	3.0	0.00021 - 0.00025	-
H. armigera	Narrabri	1/83	1927	0.00058	3.0	0.00055 - 0.00062	-
H. armigera	Emerald	3/83	1363	0.0033	1.1	0.0029 - 0.0039	5.8
H.armigera	Emerald sel.	3/83	1903	0.010	1.7	0.0082 - 0.012	17.2
H. armigera	Emerald, 2 x sel	3/83	1024	0.014	2.5	0.012 - 0.015	23.3

Third Instar

Species	Site	Date	Number	LD 50	Fiducial limits	Slope	RF
H. punctigera	Narrabri	1/83	150	0.012	0.009 - 0.014	2.5	-
H. armigera	Narrabri	1/83	150	0.02	0.01 - 0.03	3.7	-
H. armigera	Emerald	1/83	150	0.23			11.5
H. armigera	Emerald, sel	3/83	150	0.56	0.44 - 0.72	1.5	25.0
H. armigera	Emerald 2 x sel.	3./83	150	1.5	1.2 - 2.0	1.6	75

Carbamate Resistance in *H.armigera*

Introduction

The carbamates (initially carbaryl and methomyl), have been available for *H. armigera* control since the mid 1970's , but were rarely used in cotton since more effective insecticides were available. However, since the advent of pyrethroid resistance, carbamates have become an integral part of the *Helicoverpa* insecticide resistance management strategy, particularly towards the end of the season in cotton. Thiodicarb is the most widely used, it is a stomach poison andgives effective control of *Helicoverpa* when crop growth slows, but is far too expensive to be used extensively. Methomyl is a carbamate with limited residual action and it has been used against *Helicoverpa* mainly as the ovicidal component in larvicide / ovicide mixtures.

The response of *H. armigera* larvae to carbamates was monitored by this laboratory from 1983 and in this paper we report the results. We also describe techniques to bioassay thiodicarb and methomyl as an ovicide against *H. armigera.*

Materials and methods

A laboratory adapted susceptible *H. armigera* strain was obtained from Dr. R. Teakle, Department of Primary Industries Queensland, where it had been reared in laboratory conditions since 1982). *H. armigera* cultures were collected from field locations in Queensland and NSW and laboratory strains were established from at least 50 individuals. *Helicoverpa* rearing methods were described by Gunning, et. al. (1984). Insecticides were tested against the first generations of larvae reared in the laboratory of field collected strains. A methomyl resistant strain was selected by retaining the survivors of a discriminating dose ($LD_{99.9}$ for susceptibles) or higher doses.

Insecticides used were technical grade methomyl (95 %), carbaryl (99 %) and thiodicarb (93 %). Tests were also carried out with synergists piperonyl butoxide and DEF (S,S,S, - tributyl phosphorotrithioate) against the susceptible and methomyl selected strain. The larval bioassay procedures for methomyl and carbaryl, were similar to those recommeded by the Entomological Society of America . Technical grade insecticides were dissolved in acetone and five serially diluted concentrations prepared. For each concentration ten 3rd instar larvae (30 - 40 mg) were treated with one µl of solution applied by microapplicator to the dorsal thorax. Each test was replicated 3 times and every replicate included acetone treated controls.After dosage, the test larvae were held individually at 25± 1°C with adequate food and mortality was assessed 48 h. after treatment . The data were analysed by probit analysis . There was no control mortality.

Methomyl resistance frequencies were monitored from 1986 to 1990, using a discriminating dose technique (based on a dose of 1.25 µg / larva which usually resulted in 99.9 % or greater mortality of susceptibles). Resistance frequencies were monitored for stages 1, 2 and 3 of the *Helicoverpa* resistance management season . Approximately 50 cultures were collected each year from various unsprayed locations in NSW. Districts sampled were north coast, central coast, central west, and New England (Gunning and Easton, 1989).

Methomyl was bioassayed as an ovicide against *H. armigera* by the following procedure. Aqueous suspensions of methomyl E.C. (Lannate L, 22.5 %) were prepared by serial dilution and 100 24 h old, brown, fertile *H. armigera* eggs, were placed on freshly picked cotton leaves. The methomyl solutions or water only controls were sprayed onto the leaves using a Potter tower . The Potter Tower delivered a liquid deposit deposit of 1.5 mg. / cm^2. Cotton leaves with eggs were held at 25 °C in 100% humidity in petri dishes until egg hatch. After egg hatch the numbers of dead eggs and neonate larvae were counted with the aid a of a binocular microscope. Egg mortality was assessed, the data were analysed by the probit method and corrected for control mortality. Control mortality did not exceed 10%.

Thiodicarb is a stomach poison which has little contact toxicity . Bioassay required that the insecticide be incorporated into the *Helicoverpa* diet. This was achieved by dissolving thiodicarb in 2ml of formamide which was diluted to volume with a 0.5% Triton X 155 / acetone solution. Aqueous suspensions were prepared by serial dilution. Five concentrations were sprayed by Potter Tower onto a 2 mm film of *Helicoverpa* diet. After drying, the diet was cut into 1cm.squares and fed to individually maintained 30 - 40 mg larvae. Three replicates of 10 insects were treated per concentration. A water sprayed diet was prepared for each group of 10 control insects. The insects were maintained at 25 o C, in natural daylight. Mortality was assessed at 72 h and the data were analysed by the probit method, there was no control mortality.

Results

Methomyl bioassay data from 1983 - 1986 are shown in Table 6. The LD_{50}'s ranged from 0.10 - 1.7 µg / larva, with many slope values below 2.0. Based on a no survival of the susceptible $LD_{99.9}$ of , the susceptible LD_{50} range was estimated as 0.10 - 0.22 µg / larva. Most field strains tested had a proportion of individuals surviviving the discriminating dose, ranging from 5 - 62 % (Table 6). The few susceptible strains detected, were collected in 1984 . Methomyl resistance levels of the *Me Sel* strain were approximately 23 fold ,Table 7 (based on suscetible LD_{50} of 0.15 µg / larva). This strain from Emerald that was selected with methomyl, initially showed a high degree of heterogeneity with slope values of 1.2 and 1.6 for the two collections. Selection insreased the slope value to 2.6 and the LD_{50} from an average of 1.15 to 3.50. However at the LD_{99} level there was only a minor change, indicating that selection had only removed the susceptible component of the population. The *Me Sel_* strain was also resistant to carbaryl but susceptible to thiodicarb. Pre-treatment of the *Me Sel.* strain with piperonyl butoxide (PBO) and S,S.S tributyl phosphorotrithioate (DEF) showed that both (10µg PBO or 10µg DEF) synergised methomyl. Smaller synergist doses reduced the resistance levels in the *Me Sel* strain .

Resistance was not expressed by *H. armigera_* eggs of the *Me Sel* strain when methomyl was assayed as an ovicide. The LC_{50}'s of the *Me Sel* and susceptible strains were 0.0045 % (0.0035 - 0.0056)and 0.0046% (0.0039 - 0.0058) respectively, the slopes were identical at 2.8.

Since 1986/87 methomyl resistance frequencies have been monitored in the field using the discriminating dose technique. Methomyl resistant *H. armigera* were found in all areas sampled, resistance frequencies ranged from 18 to 54 %, (Fig 6). There were no significant trends in the frequency of resistance between stages of the season or year.

Bioassay results obtained for thiodicarb from 1983 - 1990 are shown in Table 8. The susceptible reference strain had an LC_{50} of 0.31 % and the slope was 3.1. LC_{50}'s of *H.armigera* field strains ranged from 0.11 - 0.45 %. There was a 4 fold variation in field strain LC_{50}'s but was unlikely to have been caused by incipient resistance. The highest LC_{50}'s were obtained in 1984, before thiodicarb was used against *H.armigera,* indicating that thiodicarb is not selecting more 'tolerant' strains.

Discussion

Methomyl and thiodicarb are an integral part of the *H. armigera* insecticide resistance management strategy . Our bioassay showed that but resistance to methomyl was widespread. From 1983 to the present 90% of strains tested have contained resistant individuals, (based on the survival of a discriminating dose). From the LD_{50}'s the resistance factors ranged from 2 to 11 fold in field *H.armigera* and low level selection increased this to 23 fold. The selected strain was also resistant to carbaryl but this has no practical significance, as carbaryl is not used against *H.armigera..* We found no cross resistance to the organophosphates. There was no evidence of any tolerance to thiodicarb, there did not appear to be any incease in LC_{50}'s with increased use over the years.

The level of methomyl resistance is sufficient to cause field failures because methomyl is a marginal chemical against *H.armigera* larvae at the best of times. (Methomyl resistance levels in *H. armigera* were probably responsible for poor control on tobacco and maize at some locations in Queensland, I.R. Kay and R.V. Gunning, unpubl. data). Methomyl resistance was not expressed by the egg stage and therefore methomyl remains an effective ovicide.

Thiodicarb is a double methomyl molecule with a sulphur atom linkage and therefore it is suprising that methomyl resistance did not confer thiodicarb resistance. The thiodicarb structure clearly reduces penetration, increases stability and may resist enzymatic attack better than methomyl.

Two carbamate resistance mechanisms are known; they may be oxidised by microsomal oxidases or the neurotransmitter acetylcholinesterases (AChE) may have decreased sensitivity to carbamate inhibition. In *H. armigera,* methomyl detoxification, perhaps by mono oxygenases and esterases, seems more likely than decreased ACh E sensitivity since we found strong synergism by PBO and DEF. Insensitive ACh Es also interfere with organophosphate action. If this occurred organophosphate cross resistance would be expected. Our results failed to show this type of resistance. The non expression of resistance by the egg stage is not surprising, since detoxification mechanisms of the embryo in eggs are probably not as well developed as in larvae.

For the *Helicoverpa* resistance management strategy, non-pyrethroid insecticides are recommended for early and late season protection. Methomyl and thiodicarb are therefore primilarily used late season, after active crop growth has ceased. The strategy attempts to reduce pyrethroid resistance and to maintain *H. armigera* susceptibility to other insecticides. The long term management of *H. armigera* resistance has been based on the assumption that limited insecticide use will preserve some susceptibity allowing some resistance dilution/reversion which will allow levels to fall by the start of the next spraying season. Forrester has shown that this has occurred with fenvalerate resistance in sprayed *H.armigera* populations every year so far, since the onset of the strategy. The yearly resistance dilution/reversion is probably not sufficient to last indefinitly because our data and that of Gunning and Easton (1989) show that there is a high degree of pyrethroid and methomyl resistance contamination in the refugia we have sampled in NSW. Both methomyl and pyrethroid resistants are distributed throught these unsprayed populations in NSW, apparently at little or no selective disadvantage.

Since the strategy started there has not been a major increase in methomyl resistance.Our data (Table 6, Fig. 6) show that resistant individuals occur at a reasonably high frequency all year with no clear evidence of a response to the strategy. It is likley that methomyl resistance has developed almost independently of the strategy ie. selection on sweet corn and tobacco etc.

Problems with increasing pyrethroid resistance and with endosulfan efficacy and environmental toxicity will inevitably increase thiodicarb use against *H.armigera.* It is encouraging that methomyl resistance does not confer cross resistance to thiodicarb and that there is no evidence of increasing tolerance. However *H.armigera* have a demonstrated ability to develop insecticide resistance and great care must be taken to ensure that *H.armigera* remain susceptible to thiodicarb by avoiding the dangers of overuse.

Table 6. Response of Helicoverpa armigera strains to methomyl. Insecticide was topically applied to 3rd instar larvae of the first lab generation.

Collection	**Date**	**Crop**	**LD 50** µg/larva	**fiducial limits**	**slope**	**Resistance#** %
Lab strain - Sus.	7/83		0.15	0.10 - 0.20	3.6	0
Emerald	3/83	cotton	0.69	0.52 - 0.91	1.9	47
Moree,NSW	4/83	cotton	0.67	0.45 - 0.99	2.2	41
Mareeba,Q	5/83	tobacco	0.28	0.23 - 0.33	3.8	0
Ormiston,Q	5/83	carnations	0.18	0.12 - 0.37	2.4	0
Bowen,Q	5/83	tomatoes	0.10	0.07 - 0.14	1.8	6
Mareeba	8/83	tobacco	0.90	0.62 - 1.41	1.6	52
Gyndie,Q	8/83	chick peas	0.27	0.19 - 0.39	2.1	8
Coolabunia,Q	9/83	titiciale	0.78	0.50 -0.12	1.7	36
Bananna,Q	9/83	wheat	0.40	0.33 - 0.56	2.5	12
Bowen	9/83	tomatoes	0.40	0.26 - 0.60	1.7	35
Gatton.Q	9/83	chick peas	0.19	0.13 - 0.25	1.9	10
Cloya,Q	9/83	chick peas	0.29	0.22 - 0.38	3.0	5
Crownthorpe,Q	9/83	triticale	0.25	0.18 - 0.34	2.3	10
Emerald	10/83	sunflower	0.30	0.24 - 0.37	2.7	6
Norwin,Q	10/83	chick peas	0.35	0.29 - 0.42	3.3	7
Nippan,Q	11/83	maize	0.19	0.12 - 0.31	1.4	0
Emerald	12/83	maize	0.23	0.16 - 0.32	2.5	6
Tamworth,NSW	1/84	light trap	0.22	0.12 - 0.15	2.6	1
Cowra,NSW	2/84	maize	0.25	0.13 - 0.33	2.8	0
Rockhampton,Q	2/84	maize	0.16	0.13 - 0.23	2.4	0
Emerald	3/84	cotton	0.44	0.28 - 0.65	1.5	17
Gyndie	3/84	sorghum	0.23	0.15 - 0.40	0.8	14
Emerald	3/84	maize	0.44	0.39 - 0.60	2.2	26
Bowen	3/84	tomatoes	0.20	0.10 - 0.36	1.3	10
Narrabri,NSW	3/84	cotton	0.36	0.25 - 0.54	2.3	16
Dalma,Q	4/84	sorghum	1.00	0.82 - 1.1	1.2	50
Emerald	4/84	maize	0.17	0.14 - 0.27	1.7	0
Nambour,Q	4/84	maize	0.21	0.18 - 0.24	1.6	5
Emerald	5/84	maize	0.31	0.21 - 0.44	1.9	22
Redland Bay	5/84	sweet corn	0.16	0.12 - 0.19	2.9	0
Darwin	6/84	maize	0.27	0.19 - 0.37	2.1	0
Biloela,Q	4/84	maize	0.24	0.19 - 0.31	2.6	0
Millaroo	7/84	maize	0.33	0.11 - 0.43	2.7	5
Clare,Q	7/84	maize	0.50	0.40 - 0.72	2.2	20
Emerald	8/84	sunflower	0.80	0.50 - 1.4	1.3	28
Emerald	8/84	sunflower	0.80	0.6 - 1.3	1.8	60
Mona Park,Q	8/84	sunflower	1.00	0.89 - 1.1	2.7	32
Darwin	7/84	pumpkin	0.12	0.08 - 0.12	1.6	0
Emerald	7/84	maize	0.15	0.10 - 0.30	1.0	27
Mareeba	9/84	tobacco	0.80	0.56 - 1.3	1.7	25
Jambin	11/84	maize	0.45	0.35 - 0.62	2.5	25
Nippan	11/84	maize	0.51	0.39 - 0.68	3.0	9
Gatton	12/84	sweet corn	0.32	0.20 - 0.45	1.4	30
Yanco,NSW	3/85	sweet corn	1.70	-	0.8	55
Bowen	5/85	tomatoes	0.80	0.49 - 1.3	2.1	41

Mareeba	5/85	lab purpeus	0.90	0.60 - 1.1	2.8	36
Capella	5/85	maize	0.68	0.52 - 0.94	3.5	27
Mareeba	8/85	tobacco	0.55	0.38 - 0.79	1.5	31
Mareeba	9/85	tobacco	0.44	0.32 - 0.59	1.9	19
Mareeba	9/85	tobacco	0.32	0.23 - 0.42	2.0	17
Kununurra.WA	9/85		0.72	0.38 - 1.4	1.4	41
Mareeba	9/85	tobacco	0.27	0.21 - 0.37	2.2	15
Gordonstone	9/85	sunflower	0.40	0.30 - 0.50	2.1	34
Jambin	9/85	chick peas	0.39	0.28 - 0.54	2.2	19
Capella	9/85	sunflower	0.42	0.33 - 0.53	2.6	17
Bowen	9/85	tomatoes	0.29	0.21 - 0.38	2.7	10
Wowan	9/85	linseed	0.72	0.54 - 0.96	2.0	34
Gordon Downs	10/85	maize	0.60	0.36 - 1.2	1.2	47
Emerald	10/85	maize	0.38	0.30 - 0.59	1.5	29
Emerald	10/85	maize	0.35	0.25 - 0.50	1.6	29
Mareeba	11/85	tobacco	0.36	0.25 - 0.53	1.7	24
Tamworth	11/85	light trap	0.36	0.29 - 0.47	2.6	18
Emerald	11/85	maize	0.36	0.29 - 0.46	2.4	20
Emerald	12/85	maize	0.65	0.47 - 0.89	2.3	36
Wowan	12/85	maize	0.45	0.29 - 0.78	1.2	44
Ormiston	12/85	sweet corn	0.31	0.16 - 0.59	0.9	37
Grafton	1/86	maize	0.36	0.20 - 0.63	1.3	34
Caroona,NSW	1/86	maize	1.20	0.50 -3.2	0.7	40
Griffith	1/86	sweet corn	0.70	0.50 - 1.0	2.2	33
Moree	1/86	maize	0.56	0.38 - 0.76	2.0	30
Breeza	2/86	light trap	0.32	0.23 - 0.42	2.0	19
Forbes,NSW	2/86	maize	0.50	0.40 - 0.70	3.0	20
Biloela	2/86	cotton	0.71	0.48 - 1.1	1.6	35
Singleton	2/86	maize	0.30	0.23 - 0.49	1.7	28
Warren,NSW	2/86	maize	0.50	0.39 - 0.61	1.7	23
Biloela	2/86	sorghum	0.72	0.56 - 1.0	1.9	31
Kingower	2/86	cotton	0.87	0.69 - 1.0	2.9	47
Emerald*	2/86	cotton	1.30	0.90 - 2.0	1.2	52
Emerald*	2/86	cotton	1.00	0.72 - 1.58	1.6	47
Emerald	3/86	cotton	0.54	0.33 - 0.90	1.9	21
Biloela	3/86	cotton	1.00	0.50 - 1.6	1.4	62
Armidale	3/86	maize	0.81	0.63 - 1.3	3.3	22
Boggabri	3/86	sunflower	0.32	0.20 - 0.50	1.7	18
Gundagai,NSW	4/86	maize	0.38	0.29 - 0.50	2.3	12
Emerald	4/86		0.29	0.20 - 0.38	2.0	8
Emerald	5/86	cotton	0.29	0.21 - 0.42	2.0	15
Bowen	5/86	tomatoes	0.26	0.18 - 0.40	1.6	10
Cowra	5/86	sweet corn	0.32	0.19 - 0.63	1.2	15
Moura	7/86	chick peas	0.47	0.34 - 0.63	2.0	28
Clare	7/86	maize	0.40	0.28 - 0.62	1.7	21
Millaroo	8/86	maize	0.40	0.27 - 0.60	1.3	30

* Survivors retained to establish a resistant strain (see Table 7).

Resistance calculated as percentage survival of the susceptible $LD_{99.9}$.

Table 7. Response of a methomyl selected strain of <u>Helicoverpa armigera</u> to three carbamates on the effects of synergists on methomyl toxicity.#

Insecticide	Strain	LD 50 (µg/larva)	fiducial limits	slope	RF*
methomyl	Lab strain - Sus.	0.15	0.10 - 0.20	3.6	-
methomyl	Emerald 2 /86	1.30	0.90 - 2.0	1.2	9
	Emerald 2/86	1.00	0.72 - 1.56	1.6	7
	Me Sel.	3.50	2.4 - 5.1	2.6	23
methomyl + Pbo 0.2 %	Me Sel.	2.90	2.3 - 4.5	2.0	19
methomyl + Pbo 0.4 %	Me Sel	0.41	0.30 - 0.61	2.1	2
methomyl + DEF 0.1 %	Me Sel.	1.00	0.85 - 1.2	2.1	7
methomyl + DEF 1.0 %	Me Sel.	0.35	0.24 - 0.49	2.1	2
carbaryl	Me Sel.	91.0	59 - 158	1.2	100
thiodicarb	Me Sel.	0.28+	0.10 - 0.55	2.3	1

Methomyl and carbaryl were topically applied to 3rd instar larvae, thiodicarb was administered via ingestion.

+ LC_{50} for thiodicarb expressed as % a.i.

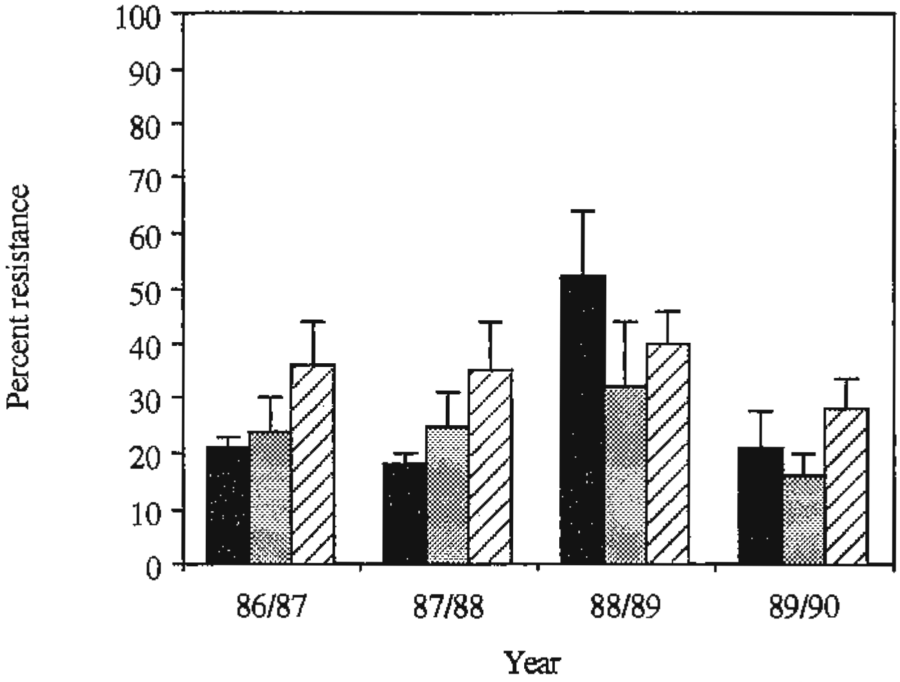
* Resistance factors were calculated as the ratio of resistant LD_{50} / susceptible LD_{50}. Carbaryl susceptible LD_{50} (0.9 µg / larva) was derived from a 1973 / 74 Bathurst strain of <u>H. armigera</u> (G. Goodyer, pers. com.).

Table 8. Response of Helicoverpa armigera to thiodicarb.#

Collection	**Date**	**Crop**	**LC $_{50}$** % a.i.	**fiducial limits**	**slope**
Lab strain - Sus.	9/93		0.31	0.28 - 0.36	3.1
Emerald,Q	4/83	cotton	0.21	0.15 - 0.30	3.8
Norwin,Q	10/83	chick peas	0.22	0.17 - 0.29	3.5
Breeza,NSw	2/84	light trap	0.14	0.10 - 0.19	3.9
Tamworth,NSW	2/84	light trap	0.45	0.40 - 0.50	3.8
Rockhampton,Q	2/84	maize	0.13	0.10 - 0.17	3.3
Cowra,NSW	2/84	sweet corn	0.11	0.07 - 0.17	2.0
Grafton,NSW	3/84	maize	0.25	0.13 - 0.38	3.3
Emerald	3/84	maize	0.44	0.23 -0.65	2.6
Gloucester,NSW	4/84	carnations	0.21	0.18 - 0.28	3.4
Capella,Q	6/85	maize	0.34	0.27 - 0.44	3.1
Gordonstone,Q	9/85	sunflower	0.19	0.13 - 0.21	2.3
Mareeba,Q	9/85	lab pupureus	0.37	0.26 - 0.51	2.3
Wowan,Q	9/85	linseed	0.16	0.10 - 0.25	2.5
Kingower,Q	2/86	cotton	0.28	0.20 - 0.44	2.3
Emerald	2/86	cotton	0.31	0.11 - 0.53	2.8
Emerald	2/86	cotton	0.48	0.30 - 0.75	2.3
Breeza,NSW	2/87	light trap	0.21	0.15 - 0.25	2.8
Tamworth,NSW	2/87	light trap	0.35	0.30 - 0.39	3.0
Bellingen,NSW	2/87	maize	0.30	0.25 - 0.33	3.2
Kempsey,NSW	2/88	maize	0.20	0.14 - 0.23	3.0
Grafton,NSW	2/88	maize	0.24	0.17 - 0.30	2.8
Tamworth	2/88	light trap	0.31	0.25 - 0.35	3.1
Breeza	2/89	light trap	0.19	0.14 - 0.21	2.4
Bellingen	2/89	maize	0.33	0.27 - 0.41	3.2
Maitland, NSW	2/89	maize	0.25	0.20 - 0.29	3.2
Narrabri,NSW	1/90	cotton	0.22	0.13 - 0.32	5.4
548	1/90	maize	0.29	0.25 - 0.35	5.9
Emerald	3/90	cotton	0.29	0.19 - 0.45	3.7

Thiodicarb was administered to 3rd instar larvae via ingestion.

Figure 6. The frequency of methomyl resistance in *Helicoverpa armigera* collected from unsprayed maize crops in NSW 1987 - 1990. Vertical bars represent standard errors. Stage 1 ■, Stage 2 ▩, Stage 3 ▨ are the stages of the *Helicoverpa* resistance management season. (Stage 2 represents the pyrethroid window, Jan 10 to Feb 20 for the first three years and Jan 10 to Feb 13 1989/90).



Organophosphorous Resistance in *H.armigera*

Introduction

Methyl parathion was frequently used to control DDT resistant *H.armigera* in the 1970's but use declined after the registrations of pyrethroids for *H.armigera* control. Organophosphates are presently used in the *Helicoverpa* resistance management strategy. Their main use is late season in Stage 3 when both pyrethroids and endosulfan are not recomended. However insect pressure can be quite variable and hence organophosphate usage. The most commonly used organophosphate is profenfos with smaller usage of parathion, sulprofos and monocrotofos also used.

H. armigera susceptibilty to organophosphorous insecticides (methyl parathion, sulpropfos and profenofos) has been monitored since 1974. This report presents the results from populations collected from locations in Queensland and NSW.

2. Materials and Methods

H. armigera cultures were collected from field locations in Queensland and NSW and laboratory generations were established from at least 50 individuals. Insecticides were tested against the first genations of larvae reared in the laboratory. Insecticides used were technical grade methyl parathion (Bayer, 99%), sulprofos (Bayer, 89.5 %), profenofos (Ciba-Geigy, 89.3 %). Technical grade insecticides were dissolved in acetone and five serially diluted concentrations prepared. For each concentration ten 3rd instar larvae (30 - 40 mg) were treated with one µl of solution applied by microapplicator to the dorsal thorax. Each test was replicated 3 times and every replicate included acetone treated controls.After dosage, the test larvae were held individually at 25^{+} 1^{o}C with adequate food and mortality was assessed 48 h. after treatment. The data were analysed by probit analysis. There was no control mortality.

A a rapid discriminating dose technique was used from 1988 to monitor sulprofos and profenofos resistance frequncies in *H. armigera* . This was based on doses of 2.0 and 1.0 µg / larva respectively, which usually resulted in 99.9 % or greater mortality of the susceptibles.Resistance frequencies were monitored for stages 1, 2 and 3 of the <u>Helicoverpa</u> resistance management season. Approximately 50 cultures were collected each year from various unsprayed locations in NSW. Districts sampled were north coast, central coast, central west, and New England (Gunning and Easton, 1989).

Results

Results obtained for methyl parathion, sulprofos and profenofos against the *H. armigera* strains from various locations are shown in Tables 9 to 11. Methyl parathion data from 1974 - 1983 is presented . The LD $_{50's}$ were within a range of 0.11 to 0.5 µg per larva. There was little interstrain variation or evidence of heterogeneity and should be consided it to be representative baseline data for methyl parathion against *H. armigera.* For profenofos, LD $_{50's}$ ranged from 0.11 - 1.2 µg per larva (a 10 fold variation) and 17% of the slope values were low . Sulprofos had a 4.5 fold range in LD $_{50's}$ (0.16 -0.71) accompanied by variable slope values (a cluster of slope values below 2.0 in 1985/1986).

Profenofos resistance frequencies monitored by the discriminating dose technique 1988/89 - 1989/90 (Fig 8) showed a low frequency (10 - 30 %) of resistants distributed in unsprayed NSW populations.Sulprofos data for the 1989/90 season (Fig. 7), showed resistance frequencies which varied from 20 - 40%.

Discussion

The data has provided baseline toxicity data for methyl parathion against *H. armigera*. These data are valuable because they provide information against which any future changes can be measured.However, more recent bioassay data for profenfos and sulprofos showed a heterogeneity of response probably caused by incipient resistance which has been detectable since 1985/86. Resistance frequencies seemed not to increase in Stage 3 and it thus seems that organophosphorous use in *Helicoverpa* resistance management strategy has not exacerbated the problem. However, great care should be taken to avoid the dangers of overuse.

Table 9. Response of Australian strains of *Helivcoverpa armigera.*to topically applied methyl parathion. Insecticide was topically applied to 3rd instar larvae of the first lab generation.

Location	Date	Crop	LD 50 µg/larva	Fiducial Limits	Slope
Nevertire, NSW	3/74	cotton	0.45	0.40 - 0.51	5.5
Wee Waa, NSW	3/74	cotton	0.39	0.25 - 0.55	3.1
Wee Waa	2/74	cotton	0.50	0.44 - 0.58	4.8
Breeza, NSW	8/74	maize	0.23	0.19 - 0.28	3.1
Wee Waa	3/74	cotton	0.37	0.30 - 0.44	2.8
Wee waa	9/74	sorghum	0.25	0.12 - 0.36	4.7
Wee Waa	19/1/75	cotton	0.22	0.12 - 0.32	4.2
Wee Waa	22/5/75	cotton	0.45	0.38 - 0.53	3.9
Narrabri, NSW	3/3/76	light trap	0.25	0.12 - 0.38	3.1
Whitton, Q	15/4/76	-	0.34	0.25 - 0.43	2.8
Mareeba,Q	6/3/76	tobacco	0.47	0.39 - 0.57	3.3
St George,Q	14/3/76	-	0.61	0.50 - 0.74	3.2
Brookstead,Q	28/3/76	-	0.39	0.32 - 0.48	3.2
Emerald,Q	20/4/76	-	0.47	0.37 - 0.61	2.7
Biloela,Q	8/3/77	-	0.30	0.25 - 0.35	3.9
Tamworth,NSW	9/5/79	cabbage	0.18	0.15 - 0.22	4.6
Katherine,NT	18/4/79	-	0.24	0.17 - 0.32	2.3
Brookstead,Q	5/3/80	cotton	0.18	0.15 - 0.21	4.3
St George	5/4/80	cotton	0.26	0.21 - 0.31	4.2
Wee Waa	26/4/80	cotton	0.25	0.20 - 0.31	3.1
Griffith,NSW	11/6/80	-	0.19	0.15 - 0.24	3.2
Tamworth	1/81	maize	0.23	0.19 - 0.26	4.4
Taree,NSW	31/3/81	maize	0.19	0.15 - 0.24	3.2
Maclean	4/81	maize	0.14	0.12 - 0.17	5.0
Grafton,NSW	4/81	maize	0.17	0.14 - 0.19	4.0
Moree,NSW	13/5/81	maize	0.41	0.35 - 0.48	3.5
Brookstead	5/81	cotton	0.22	0.17 - 0.27	3.1
Gt George	5/81	cotton	0.29	0.21 - 0.38	2.3
Kununurra,WA	19/8/81	sunflower	0.11	0.09 - 0.13	5.6
Quirindi,NSW	25/2/82	sunflower	0.27	0.15 - 0.40	3.4
Grafton	26/3/82	maize	0.29	0.24 - 0.36	3.6
Kununurra	11/3/82	sumflower	0.49	0.41 - 0.57	4.3
Cecil Plains,Q	18/3/82	maize	0.44	0.36 - 0.55	3.6
Narrabri	29/4/82	light trap	0.29	0.24 - 0.36	3.8
Emerald	5/82	cotton	0.44	0.36 - 0.52	3.7
Emerald	12/82	cotton	0.44	0.36 - 0.52	3.2
Narrabri	27/1/83	light trap	0.17	0.14 - 0.21	3.5
Kununurra	25/1/83	sunflower	0.18	0.15 - 0.21	5.2
Brookstead	21/2/83	cotton	0.50	0.41 - 0.60	3.5
Biloela	3/83	maize	0.31	0.26 - 0.37	4.3

Table 10. Response of strains of Australian *Helicoverpa armigera* to topically applied sulprofos.Insecticide was topically applied to 3rd instar larvae of the first lab generation.

Location	**Date**	**Crop**	**LD 50 µg/larva**	**Fiducial limits**	**Slope**
Emerald, Q	12/82	cotton	0.38	0.14 - 0.60	3.3
Bowen,Q	5/93	tomatoes	0.50	0.38 - 0.62	2.6
Ormiston,Q	5/83	carnations	0.38	0.29 - 0.47	2.4
Gyndie,Q	20/3/84	sorghum	0.38	0.31 - 0.45	3.5
Emerald	21/3/84	maize	0.32	0.21 - 0.44	2.4
Biloela,Q	27/4/84	cotton	0.59	0.36 - 0.73	1.9
Emerald	11/4/84	cotton	0.33	0.27 - 0.40	5.4
Redland, Q	16/5/84	sweet corn	0.49	0.39 - 0.61	4.2
Darwin, NT	17/7/84	pumpkin	0.36	0.30 - 0.43	3.9
Millaroo, Q	27/7/84	maize	0.47	0.37 - 0.58	4.5
Mareeba, Q	30/5/85	lab purpeus	0.79	0.59 - 0.98	3.5
Capella, Q	26/6/85	maize	0.54	0.38 - 0.70	2.2
Mareeba	8/85	tobacco	0.59	0.47 - 0.72	2.9
Mareeba	9/85	tobacco	0.50	0.38 - 0.62	2.5
Orion, Q	8/5/85	maize	0.89	0.72 - 1.10	2.8
Mareeba	9/85	tobacco	0.40	0.30 - 0.50	2.6
Kununurra, WA	9/85	sunflower	0.60	0.43 - 0.77	2.0
Gordonstone, Q	4/9/85	sunflower	0.50	0.40 - 0.60	3.2
Capella, Q	14/9/85	sunflower	0.40	0.17 - 0.64	1.8
Emerald	23/10/85	maize	0.39	0.29 - 0.50	2.3
Emerald	28/10/85	maize	0.48	0.38 - 0.60	2.2
Jambin, Q	11/9/85	chick peas	0.46	0.30 - 0.62	2.4
Emerald	14/11/85	maize	0.50	0.40 - 0.60	2.6
Emerald	3/12/85	maize	1.2	0.72 - 1.48	1.9
Wowan, Q	17/12/85	maize	0.62	0.44 - 0.80	2.1
Grafton, NSW	16/1/86	maize	0.80	0.41 - 1.2	1.4
Warren, NSW	24/2/86	maize	0.31	0.20 - 0.41	2.1
Breeza, NSW	4/2/86	light trap	0.44	0.31 - 0.58	2.7
Singelton, NSW	20/2/86	maize	0.38	0.29 - 0.48	3.0
Kingower, Q	27/2/86	cotton	0.46	0.32 - 0.59	3.6
Emerald	18/3/86	cotton	0.68	0.53 - 0.83	3.5
Emerald	12/3/86	light trap	0.56	0.43 - 0.70	2.4
Boggabri, NSW	26/3/86	sunflower	0.32	0.23 - 0.43	2.4
Emerald	30/4/86	maize	0.46	0.36 - 0.57	3.1
Bowen	8/5/86	tomatoe	0.36	0.28 - 0.44	2.3
Biloela	17/7/86	light trap	0.30	0.22 - 0.40	2.8
Spring Ridge,NSW	17/12/86	maize	0.47	0.35 - 0.60	2.2
Narromine	8/1/87	maize	0.46	0.34 - 0.59	2.4
Carrol, NSW	21/2/87	maize	0.67	0.51 - 0.83	2.6
Mudgee, NSW	11/2/87	maize	0.32	0.20 - 0.44	2.3
Breeza	10/2/87	light trap	0.36	0.29 - 0.43	3.3
Mudgee	15/2/87	maize	0.29	0.16 - 0.42	2.0
Miles, Q	15/2/87	maize	0.18	0.10 - 0.26	1.5
Thangool, Q	24/2/87	maize	0.62	0.44 - 0.80	2.4
Killarney,Q	14/2/87	maize	0.50	0.36 - 0.64	2.3
Thangool	19/2/87	-	0.33	0.24 - 0.44	1.6
Norwin,Q	13/3/87	-	0.11	0.05 - 0.17	1.9
Brigalow,Q	3/4/87	-	0.15	0.07 - 0.23	1.2
Tamworth,NSW	6/10/87	light trap	0.31	0.24 - 0.38	2.3
Bowen	7/10/87	tomatoe	0.29	0.15 - 0.43	2.1
Bellingen, NSW	2/12/87	maize	0.55	0.40 - 0.70	3.1
Gatton,Q	12/87	cotton	0.26	0.12 - 0.40	2.3
Maitland, NSW	9/12/87	maize	0.58	0.46 - 0.72	4.0
Breeza	16/2/88	light trap	0.45	0.22 - 0.77	1.6

Table 11. Response of strains of Australian *Helicoverpa armigera* to topically applied profenofos.Insecticide was topically applied to 3rd instar larvae of the first lab generation.

location	Date	Crop	LD 50 µg/larva	fiducial limits	Slope
Brookstead,Q	3/80	cotton	0.20	0.17 - 0.23	5.6
Emerald,Q	4/83	cotton	0.31	0.23 - 0.39	3.1
Emerald	12/83	sunflower	0.55	0.42 - 0.68	2.6
Nippan,Q	12/83	-	0.18	0.10 - 0.26	3.5
Nandi,Q	15/2/84	-	0.18	0.13 - 0.24	3.5
Emerald	21/3/84	maize	0.40	0.33 - 0.47	3.3
Emerald	11/4/84	cotton	0.21	0.16 - 0.27	2.8
Biloela,Q	27/4/84	cotton	0.33	0.23 - 0.43	2.4
Gyndie,Q	6/84	sorghum	0.20	0.15 - 0.25	2.5
Redland Bay,Q	16/5/84	sweet corn	0.24	0.17 - 0.31	2.5
Darwin,NT	17/7/84	pumpkin	0.16	0.13 - 0.20	3.7
Emerald	7/84	maize	0.28	0.17 - 0.39	2.1
Millaroo,Q	27/7/84	maize	0.34	0.26 - 0.46	2.6
Mareeba,Q	4/9/84	tobacco	0.30	0.20 - 0.30	3.4
Emerald	1/11/84	sorghum	0.37	0.28 - 0.46	2.8
Jambin,Q	12/84	maize	0.19	0.14 - 0.23	2.7
Orion,Q	9/5/85	maize	0.25	0.18 - 0.31	3.1
Emerald	14/1185	maize	0.16	0.12 - 0.20	1.7
Mona Park,Q	24/5/85	sweet corn	0.36	0.29 - 0.43	3.9
Mareeba	30/5/85	tobacco	0.17	0.11 - 0.24	1.9
Capella,Q	26/6/85	maize	0.30	0.21 - 0.40	2.0
Mareeba	8/85	tobacco	0.27	0.23 - 0.31	3.1
Mareeba	9/85	tobacco	0.26	0.21 - 0.31	3.3
Gordonstone,Q	4/9/85	sunflower	0.20	0.15 - 0.26	2.5
Jambin,Q	11/9/85	chick peas	0.21	0.17 - 0.25	2.7
Mareeba	9/85	tobacco	0.24	0.20 - 0.28	4.6
Emerald	28/10/85	maize	0.29	0.21 - 0.37	2.5
Emerald	3/12/85	maize	0.49	0.31 - 0.67	1.5
Wowan,Q	17/12/85	maize	0.43	0.19 - 0.67	1.0
Warren,NSW	24/2/86	maize	0.15	0.12 - 0.18	1.2
Kingower,Q	29/2/86	cotton	0.30	0.20 - 0.40	2.9
Griffith,NSW	20/1/86	sweet corn	0.36	0.30 - 0.42	1.0
Kempsey,NSW	27/11/86	maize	0.18	0.10 - 0.26	2.1
Narromine	10/12/86	maize	0.22	0.10 - 0.32	2.5
Bowen,Q	8/5/86	tomatoes	0.19	0.13 - 0.25	1.8
Emerald	17/7/86	maize	0.15	0.13 - 0.17	3.0
Emerald	6/86	-	0.22	0.13 - 0.29	1.9
Spring Ridge,NSW	17/12/86	maize	0.40	0.32 - 0.49	3.0
Narromine,NSW	8/1/87	maize	0.51	0.39 - 0.63	2.8
Jerrys Plains,NSW	15/1/87	maize	0.71	0.61 - 0.82	3.2
Breeza,NSW	27/1/87	light trap	0.18	0.12 - 0.25	3.1
Carrol,NSW	21/1/87	maize	0.49	0.39 - 0.59	2.9
Mudgee,NSW	11/2/87	maize	0.29	0.16 - 0.44	2.0
Norwin,Q	13/3/87	-	0.17	0.13 -0.21	3.7
Kununurra	24/7/87	maize	0.19	0.14 - 0.25	3.9
Bowen	18/6/87	tomatoes	0.19	0.12 - 0.26	2.9

Figure 7 The frequency of sulprofos resistance in Helicoverpa armigera collected from unsprayed maize crops in NSW 1987 - 1990. Vertical bars represent standard errors. Stage 1 ■, Stage 2 ▒, Stage 3 ▨ are the stages of the Helicoverpa resistance management season. (Stage 2 represents the pyrethroid window, Jan 10 to Feb 20 for the first three years and Jan 10 to Feb 13 1989/90).

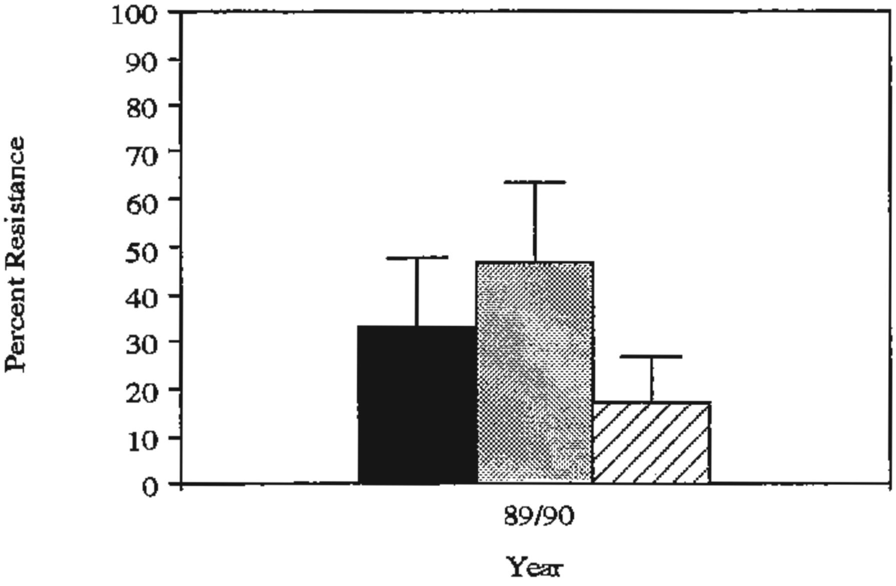
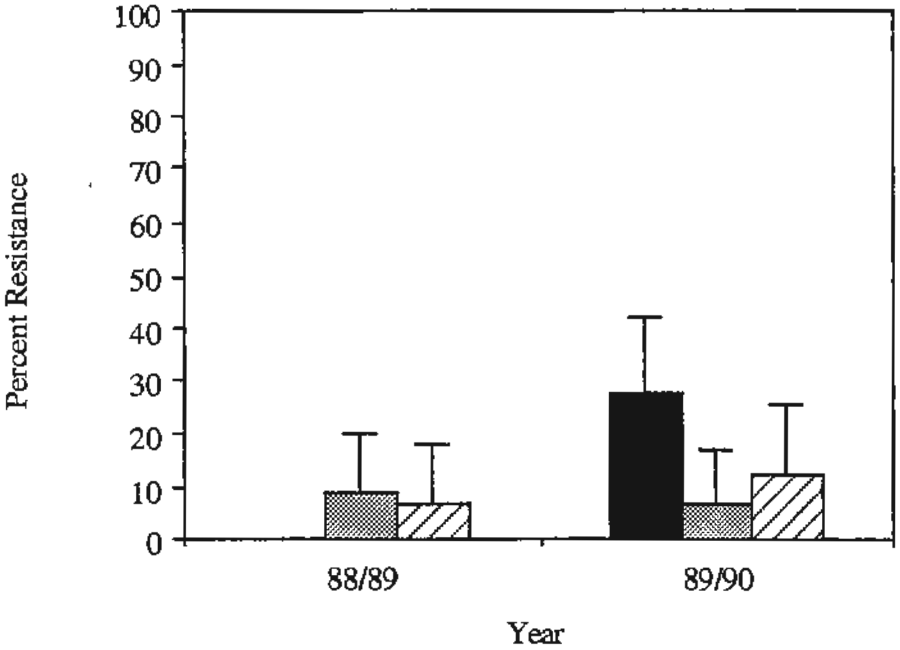


Figure 8 The frequency of profenofos resistance in *Helicoverpa armigera* collected from unsprayed maize crops in NSW 1989/90. Vertical bars represent standard errors. Stage 1 ■, Stage 2 ▒, Stage 3 ▨ are the stages of the *Helicoverpa* resistance management season (Stage 2 represents the pyrethroid window Jan10 to Feb 13).



Endosulfan Resistance in *H.armigera*

Endosulfan has been used to contol *H. armigera* on cotton and other crops since the early 1970's. There have been some past reports of endosulfan tolerance in Queensland. Endosulfan was commonly used against DDT resistant *H.armigera*, but its use declined after the first registration of pyrethroid insecticides in 1978. Since 1983/84, when the *Helicoverpa* resistance management strategy was instituted, endosulfan has been the most widely used insecticide on cotton and other summer crops. Endosulfan has been an integral part of the resistance management strategy. To prevent overuse, endosulfan is only recommended for use in early and mid-season.

Endosulfan susceptibility levels in Australian *H. armigera* have been monitored at Tamworth since 1974.

Materials and Methods

A laboratory adapted susceptible *H. armigera* strain was obtained from Dr. R. Teakle, Department of Primary Industries Queensland, where it had been reared in laboratory conditions since 1982). *H. armigera* cultures were collected from field locations in Queensland and NSW and laboratory strains were established from at least 50 individuals. *Helicoverpa* rearing methods have been described by Gunning, *et. al.* (1984). Insecticides were tested against the first generations of larvae reared in the laboratory. Endosulfan resistant cultures were selected by retaining the survivors of a discriminating dose ($LD_{99.9}$, 10 µg or greater) and rearing them to produce a endosulfan selected generation.

Insecticides used were technical grade endosulfan (80%). The larval bioassay procedures were similar to those recomeded by the Entomological Society of America. Technical grade insecticides were dissolved in acetone and five serially diluted concentrations prepared. For each concentration ten 3rd instar larvae (30 - 40 mg) were treated with one µl of solution applied by microapplicator or a micropippette, to the dorsal thorax. Each test was replicated 3 times and every replicate included acetone treated controls.After dosage, the test larvae were held individually at 25± 1°C with adequate food and mortality was assessed 48 h. after treatment. The data were analysed by probit analysis. There was no control mortality.

Since 1986, as part of a study to determine the resistance status of unsprayed *H.armigera* populations in NSW (Gunning and Easton, 1989), approximately 50 cultures were collected each year from various unsprayed locations in NSW. Districts sampled were north coast, central coast, central west, and New England. These locations were in a semicircle around the insecticide sprayed Namoi/Gwydir cotton irrigation area in northern NSW, at least 300 Km distant. Endosulfan resistance frequencies were monitored using a discriminating dose technique. This more rapid technique was based on a dose of 12.5 µg / larva which resulted in 100% mortality of susceptibles. The endosulfan resistance frequency was monitored for each strain collected. The data were pooled and results were expressed as the mean resistance frequency (+ S.E.) for stage 1 (early season), stage 2 (middle season) and stage 3 (late season) of the *Helicoverpa* resistance management year. The frequency of endosulfan resistant individuals from the sprayed Namoi/Gwydir cotton and from Emerald, Q were monitored concurrently at Narrabri, NSW (Forrester, unpublished).

Results

Endosulfan bioassay data are shown in Table. 12. From 1974 -1977 in NSW, the *H.armigera*_ cultures showed a high degree of heterogeneity, slope values were low and did not exceed 2.0. There were some very highly resistant populations indicated by resistance factors as high as 49.6-fold and up to 94 % survival of the discriminating dose.However the high resistance levels were not uniformly distributed across all sampling locations, with one 1977 collection (Moore Creek)recording a resistance factor of 2.7 and only 8% survival of the discriminating dose.

Between 1979 and early 1983 the endosulfan LD_{50}'s fell, ranging from 0.6 - 2.6 µg/larva. Slope values from 2.5 - 3.8 indicated little heterogeneity. There was negligiable survival of the discriminating endosulfan dose, only 3% survival was recorded in 2 out of 16 strains tested.

There was no effective difference in response to endosulfan between these cultures and the susceptible strain. Susceptible *H.armigera* were found in both NSW and Queensland.

From 1984 onward *H.armigera* strains again demonstrated endosulfan resistance. While the LD_{50}'s varied from 1.4 to 25.0 µg/larva, the slope values (seldom exceeding 2.0) showed a high degree of heterogeneity. Resistance factors were low seldom exceededing 10-fold, but in two the field strains tested were as high as 23 times that of susceptible strain . However, in almost all strains tested considerable proportions of individuals survived the discriminating dose(>70% in the most resistant strains). During this time only 5 of the 83 strains tested could be considered susceptible (based on 5% or lessser survival of the discriminating dose). There were similar frequencies of endosulfan resistant individuals from both NSW and Queensland. It was not the intention of this work to study the effect of crop on resistance levels because *H.armigera* were collected wherever they could be found. Collections were made mainly from maize and cotton crops because *H.armigera* were usually more abundant on them than other crops. While cotton crops were usually sprayed and maize was not, there were no obvious effects of crop on resistance levels.

The strain from Emerald selected with endosulfan showed a high degree of heterogeneity. Selection with twice the discriminating dose (25µg/larva) increased the slope value to 1.6. and the LD_{50} from 25 to 180 µg/larva but clearly only removed the susceptible component of the population.

Since the 1986 -87 season, to determine the resistance status of unsprayed *H.armigera* , in NSW, endosulfan resistance was monitored using the more rapid discriminating dose technique. From maize crop locations (north coast, central coast, central west, and New England). at least 300 Km from the insecticide sprayed Namoi/Gwydir cotton irrigation area in northern NSW, endosulfan resistant *H.armigera* were found in all areas sampled (Fig 9). Resistance frequencies, expressed in stages 1,2 and 3 of the *Helicoverpa* resistance management season, ranged from 15 to 40%. There were no significant trends in the frequency of resistance between the stages of the *Helicoverpa* resistance management season. However, there is evidence of a slight increase in the frequency of endosulfan resistant individuals from 1986 to 1990.

Discussion

Endosulfan is an integral part of the *Helicoverpa* resistance management strategy. Our bioassay data show that Endosulfan resistance in Australian *H.armigera* from 1974 to the present is clearly related to the endosulfan use patterns during that time. *H.armigera* resistance to endosulfan in the early 1970's, such as our 1974 - 1978 data , may have been due to 10 years exposure to low rates of endrin. Endrin had been used on cotton to control another pest *Earias huegeli* (the rough bollworm). Endosulfan resistance in the early 1970's was doubtlessly exacerbated by the heavy use of endosulfan to control DDT resistant *H.armigera* and endosulfan field failures occurred in the Namoi Valley at this time.

From 1979 - 1983, we found that resistance decreased and *H.armigera* were virtually susceptible to endosulfan. This period corresponded to the introduction and extensive use of pyrethroids for control of *H.armigera* on cotton and other crops. Initially, all *H.armigera* , including endosulfan resistants, were susceptible to pyrethroids (Gunning, *et al* 1984) and this greatly reduced the endosulfan resistance gene frequency . Gunning, *et al* (1990) reported a similar decline in *H.armigera* DDT resistance, attributable to pyrethroid use.The increase of endosulfan resistance in *H.armigera* after 1983 may be attributed to the fact that endosulfan has been the most commonly used insecticide since pyrethroid resistance was confirmed at about that time. Over 50 % of *Helicoverpa* control sprays on cotton comprise endosulfan.

Following the diagnosis of pyrethroid resistant *H. armigera* (Gunning *et al*, 1984), a resistance management strategy was instituted in 1984 . Non-pyrethroid insecticides are recommended for early and late season cotton and other summer crop protection. Endosulfan is primilarly used early season (Stage 1) and as well in mid season (Stage 2). In late season(Stage 3) endosulfan is not recommended for cotton but has been used on other summer crops. The long term management of *H. armigera* resistance to pyrethroids has been based on the assumption that limited insecticide use will preserve some susceptibity and allow some resistance dilution or reversion of resistance to occur before the start of the next spraying season. Forrester .has shown that this has occurred with fenvalerate resistance in sprayed *H.armigera* populations every year so far since the onset of the strategy. Dilution or reversion must not be sufficient to extend endosulfan use indefinitly because our data and that of Gunning and Easton (1989) show that there is extensive pyrethroid, methomyl (Gunning, submitted) and endosulfan resistance contamination in the refugia sampled in NSW. Resistant individuals are distributed throughout these unsprayed populations in NSW, apparently at little or no selective disadvantage.

When the strategy started in 1984 there was a major increase in endosulfan resistance Our data (Table 12, Fig. 9) showed that resistant individuals occured at a reasonably high frequency all year round. Subsequent monitoring of resistance levels by the discriminating dose method, from both sprayed (Forrester, unpublished) andunsprayed populations have shown that endosulfan resistance levels are similar and slowly increasing. The selection of *H.armigera* to high rersistance levels in the laboratory indicates these strains could become highly resistant in the field but the strategic use of endosulfan on *H.armigera* has obviously, so far, has limited resistance selection and the current levels of resistance are still below the 1974 levels.

Despite endosulfan resistance levels, field control of *H.armigera* with endosulfan has usually been adequate. However, in the1989/90 season endosulfan gave unreliable control against *H. armigera* in Queensland. Our laboratory data suggest that increased selection with endosulfan could greatly increase resistance levels (>150-fold for laboratory selection.). To further lessen selection pressure 1990/91 endosulfan use on cotton and other crops has been further restricted in mid and late summer.

Table 12 Response of field collected strains of H. armigera to endosulfan.#

Location	Date	Crop	LD 50 (µg/larva)	fiducial limits 95%	Slope	R.F^{+}	% R^{++}
Warren,NSW	3/74	cotton	5.4	2.5 - 8.4	1.1	4.9	25
Wee Waa, NSW	3/74	cotton	4.0	2.3 - 6.9	1.5	3.6	20
Wee Waa	3/74	cotton	51.4	7.2 - 365	0.98	46.7	94
Narrabri,NSW	7/74	cotton	54.6	24.9 -119	1.1	49.6	70
Breeza,NSW	9/74	maize	26.0	16.2 - 36.1	1.3	23.6	80
Wee waa	1/75	cotton	36.0	24.6 - 52.6	2.0	32.7	70
Moore Creek, NSW	2/77	tobacco	3.0	2.0 - 4.5	1.8	2.7	8
Brookstead,Q	2/79	maize	0.9	0.3 -1.5	1.3	1.0	0
Emerald,Q	2/79	cotton	2.7	1.8 - 4.1	2.5	2.5	3
Wee waa	6/80	cotton	0.6	0.4 - 0.8	3.2	0.5	0
Brookstead	2/80	maize	1.9	1.6 - 2.4	3.2	1.7	0
St. George,Q	2/80	cotton	1.5	1.2 - 1.9	2.5	1.4	3
Emerald	2/80	cotton	2.9	2.3 - 3.5	2.5	2.6	0
Moree,NSW	5/81	maize	1.4	1.1 - 1.8	2.5	1.3	0
Brookstead	5/81	maize	1.2	1.0 - 1.5	3.2	1	0
St. George	5/81	cotton	1.2	1.0 - 1.5	3.2	1	0
Cecil Plains,Q	3/82	maize	1.7	1.4 - 2.2	3.0	1.6	0
Narrabri	4/82	cotton	1.1	0.87 - 1.3	2.9	1	0
Brookstead	2/83	cotton	2.1	1.7 - 2.6	3.6	1.9	0
Narrabri	1/83	cotton	1.7	1.3 - 2.2	2.5	1.6	0
Biloela,Q	3/83	maize	1.7	1.8 - 2.9	2.6	1.6	0
Jambin,Q	10/84	maize	9.8	6.6 - 15.0	1.8	8.9	45
Goovigen,Q	10/84	maize	1.4	0.4 - 7.2	1.2	1.3	16
Wowan.Q	10/84	sunflower	9.7	6.0 - 11.6	1.6	8.8	42
Bowen,Q	10/84	tomatoes	12.6	9.5 - 17.0	2.2	11.5	48
Emerald	10/84	safflower	2.6	1.2 - 8.5	0.9	2.4	20
Tamworth,NSW	11/84	light trap	4.2	3.2 - 5.6	3.6	3.8	3
Emerald	11/84	sorghum	6.6	4.1 - 11.0	1.5	6.0	36
Jambin	11/84	maize	7.8	5.8 - 11.0	2.5	7.1	35
Biloela	11/84	sweet corn	3.3	1.4 - 5.5	0.8	3.0	32
Moree	11/84	maize	10.0	5.8 - 16.0	1.0	9.1	52
Moree	12/84	maize	3.7	1.4 - 6.0	1.9	3.4	20
Narromine,NSW	12/84	maize	8.1	5.5 - 11.0	1.5	7.4	33
Nowra,NSW	1/85	maize	4.1	3.3 - 5.0	3.4	4.0	8
Atherton,Q	1/85	maize	4.5	2.0 - 7.1	1.4	4.1	38
Breeza	1/85	sweet corn	4.7	2.6 - 8.3	1.6	4.3	27
Formatin,Q	1/85	cotton	6.6	4.7 - 7.8	1.9	6.0	40
Emerald	1/85	cotton	3.5	2.5 - 4.8	2.6	3.2	6
Biloela	1/85	cotton	1.9	2.3 - 10.0	1.2	2.1	22
Brookstead	2/85	cotton	4.9	2.8 - 8.7	1.5	4.5	38
Orion,Q	2/85	sorghum	4.4	3.2 - 6.0	2.3	4.0	20
Breeza	3/85	Light trap	8.5	5.2 - 13.0	1.8	8.0	45
Yanco,NSW	3/85	sweet corn	5.0	3.8 - 6.9	2.3	4.6	25
Kingthorpe,Q	4/85	pigeon peas	4.7	3.5 - 6.3	2.4	4.3	17
Mona Park,Q	5/85	sweet corn	7.4	5.5 - 9.8	2.8	6.8	38
Mareeba,Q	5/85	tobacco	5.8	4.0 - 7.8	1.8	5.3	24
Capella,Q	5/85	maize	6.5	4.4 - 8.6	1.5	6.0	39
Orion	5/85	maize	8.0	5.0-11.0	1.3	7.3	42
Mareeba	5/85	tobacco	8.6	5.5 - 11.6	1.2	7.8	45

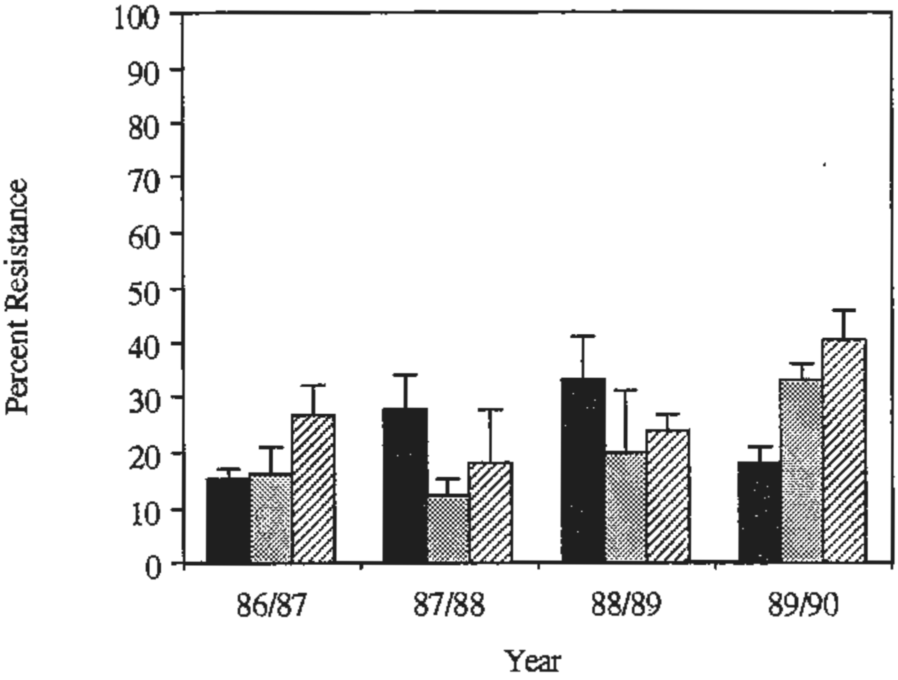
Brookstead	6/85	maize	4.4	2.0 - 7.0	2.2	4.0	13
Bowen	5/85	tomatoes	4.9	3.7 - 6.2	2.5	4.6	7
Mareeba	8/85	tobacco	3.9	3.0 - 4.9	2.1	4.0	11
Capella	5/85	sunflower	7.4	4.2 - 10.8	1.1	6.7	48
Mareeba	9/85	tobacco	3.2	1.4 - 5.3	0.8	2.9	37
Mareeba	9/85	tobacco	2.0	1.3 - 2.9	1.8	1.8	7
Jambin	9/85	chick peas	5.3	3.8 - 7.	2.0	4.8	29
Mareeba	9/85	tobacco	3.3	2.0 - 5.1	1.3	3.0	21
Wowan	9/85	Linseed	9.1	5.3 - 13.0	1.4	8.3	41
Mareeba	9/85	tobacco	4.0	2.7 - 5.8	2.1	3.6	14
Clare,Q	9/85	maize	5.0	4.0 - 6.3	1.4	4.6	41
Gordonstone.Q	9/85	sunflower	6.5	5.0 - 8.5	2.2	5.9	33
Capella	9/85	sunflower	9.3	6.5 - 12.4	1.5	8.5	46
Bowen	9/85	tomatoes	7.5	5.0 - 10.0	1.5	6.8	37
Emerald	9/85	maize	8.9	6.8 - 11.4	3.0	8.1	26
Emerald	10/85	maize	4.9	3.5 - 6.9	1.5	4.5	14
Emerald	10/85	maize	4.9	2.2 - 9.1	1.9	4.5	24
Mareeba	10/85	tobacco	4.7	3.1 - 6.9	1.7	4.3	21
Gordon Downs	10/85	maize	6.8	4.7 - 9.8	1.3	6.2	32
Gordon Downs	10/85	maize	8.0	5.1 - 10.9	2.0	7.3	43
Tamworth	11/85	light trap	3.6	2.3 - 5.4	2.2	3.3	2.2
Emerald	11/85	maize	1.9	1.4 - 5.2	0.74	1.7	33
Ormiston	12/85	sweet corn	2.6	0.5 - 5.0	0.52	2.4	42
Mareeba	12/85	tobacco	7.7	5.1 - 9.8	2.1	7.0	33
Jambin	12/85	maize	1.9	0.4 - 2.4	0.90	1.7	11
Tenterfield	1/86	maize	8.5	2.5 - 14.8	0.53	7.3	39
Pine ridge	1/86	sunflower	2.6	1.9 - 3.6	2.5	2.4	6
Tamworth	1/86	light trap	3.9	1.0 - 7.0	0.5	3.6	26
Moree	1/86	maize	2.5	1.2 - 4.0	1.6	2.3	19
Cowra,NSW	1/86	sweet corn	10.0	5.0 - 15.2	0.49	9.1	34
Grafton	1/86	maize	1.2	0.4 - 2.0	1.0	1	17
Darlington Pt,NSW	1/86	sweet corn	7.6	3.5 - 11.7	0.76	7.0	50
Warren,NSW	2/86	cotton	6.1	4.4 - 7.7	0.90	5.6	24
Kingower	2/86	cotton	9.6	5.0 - 15.0	0.8	8.7	60
Emerald	2/86	cotton	25.0	16.0 - 40.0	1.4	22.7	26
Killarney	2/6	sorghum	3.2	1.0 - 5.4	0.9	2.9	26
Singleton,NSW	2/86	maize	6.2	2.5 - 11.0	0.7	5.6	30
Forbes.NSW	2/86	maize	6.2	3.6 - 10.0	1.1	5.6	50
Emerald*	2/86	cotton	25.0	16.1 - 34.2	1.4	22.7	72
Tamworth	2/86	light trap	3.9	0.6 - 7.2	0.5	3.6	26
Tenterfield	3/86	maize	2.0	1.2 - 3.1	2.1	1.8	5
Biloela	3/86	cotton	3.6	2.3 - 5.7	1.6	3.3	19
Boggabri,NSW	3/86	sunflower	3.6	2.3 - 5.7	1.9	3.3	14
Armidale,NSW	3/86	maize	1.6	0.7 - 2.5	2.1	1.5	4
Gundagai,NSW	4/86	sweet corn	16.0	4.0 - 30.3	0.90	14.6	65
Emerald	4/86	cotton	5.4	2.8 - 8.1	1.2	4.9	45
Biloela	5/86	maize	13.0	7.4 - 19.0	1.2	11.9	38
Emerald	5/86	cotton	5.4	3.6 - 8.0	1.5	4.9	31
Bowen	5/86	tomatoes	6.8	4.7 - 9.8	1.3	6.2	47
Moura,Q	7/86	chick peas	10.0	6.9 - 16.0	1.3	9.1	52
Millaroo,Q	8/86	maize	3.6	1.7 - 5.7	1.2	3.3	26
Emerald	12/86	maize	3.0	2.0 - 3.4	1.9	2.7	15
Wowan,Q	12/86	maize	2.2	1.2 - 5.6	0.8	2	24
Breeza	12/86	light trap	3.2	1.3 - 5.5	0.60	2.9	24
Susceptible	9/83		1.1	0.9 - 1.3	3.2	1.0	-
Emerald (sel)*	4/86		125.0	21-660	0.62	113.6	-
Emerald 2xDD sel.	4/86		180.0	150-210	1.6	163.6	-

* Survivors retained to establish resistant strains.

Insecticide was topically applied to 3rd instar larvae of the first laboratory generation.
+ LD_{50} of field strains / LD_{50} of susceptible reference strain.

++ Percent survival of a discriminating dose of endosulfan (12.5 µg/larva).

Figure 9. The frequency of endosulfan resistance in Helicoverpa armigera collected from unsprayed maize crops in NSW 1986 - 1990. Vertical bars represent standard errors. Stage 1 ■, Stage 2 ▒, Stage 3 ▨ are the stages of the Helicoverpa resistance management season. (Stage 2 represents the pyrethroid window, Jan 10 to Feb 20 for the first three years and Jan 10 to Feb 13 1989/90).



Response of *Helicoverpa punctigera* to Insecticides

Introduction.

Helicoverpa punctigera are major crop pests in northern New South Wales and Queensland.*H.punctigera* are poyphagous, attacking a diverse range of plant species but appears to be restricted to dicoyledonous hosts.(*H.armigera* attacks both dicots and monocots).

H.punctigera is an endemic species which ocurrs throught the continent and is highly mobile with mass migrations from inland following sensecence of winter annuals. The moths then follow the progressive growth of spring annuals in south and eastern Australia, where the extensive cropping areas are located. By comparison *H.armigera* in Australia are more sedentary and associated with cropping areas.

Since 1974 a range of insecticides have beeen used to control *H.punctigera* on cotton and other summer crops. They were : DDT, endosulfan, carbaryl and methyl parathion in the early 1970's ; pyrethroids from 1978 to the present. Endosulfan has been the most commonly used insecticide against *H.punctigera*. Since 1983 insecticide use against both *Helicoverpa* species has been restricted by an insecticide resistance management strategy.

The response of *H.punctigera* to insecticides has been monitored in this laboratory since 1974.

Methods and Materials

H. punctigera cultures were collected from field locations in Queensland, NSW, Victoria and South Australia and laboratory strains were established from at least 50 individuals. *Helicoverpa* rearing methods were described by Gunning, *et. al.* (1984). Insecticides were tested against the first generations of larvae reared in the laboratory.

Insecticides used were technical grade endosulfan , DDT , deltamethrin , fenvalerate , methomyl , carbaryl and methyl parathion . The larval bioassay procedures were similar to those recomeded by the Entomological Society of America . Technical grade insecticides were dissolved in acetone and five serially diluted concentrations prepared. For each concentration ten 3rd instar larvae (30 - 40 mg) were treated with one µl of solution applied by microapplicator or a micropippette, to the dorsal thorax. Each test was replicated 3 times and every replicate included acetone treated controls.After dosage, the test larvae were held individually at 25± 1°C with adequate food and mortality was assessed 48 or 72 h. after treatment . The data were analysed by probit analysis. There was no control mortality.

Results

Bioassay results for the response of *H.punctigera* to DDT, endosulfan, carbaryl, methomyl and methyl parathion from 1974 to 1988 are shown in Table 13.The DDT LD_{50}'s ranged from 1.5 - 2.8 µg/larva. There was little interstrain variation or evidence of heterogeneity since slope values were 2.0 or greater. Between 1975 and 1987 LD_{50}'s for endosufan ranged from 0.5 - 1.7 µg/larva and high slope values between 2.2 and 5.6 showed little heterogeneity. Methyl parathion LD_{50}'s were between 0.14 and 0.22 µg/larva and showed no evidence of heterogeneity.Response to carbaryl and methomyl were each only checked on two occasions. In the two populations sampled for response to carbaryl a high degree of heterogeneity was indicated by low slope values (1.4 and 1.6).

Bioassay data obtained for pyrethroids (fenvalerate and deltamethrin) against *H.punctigera* are shown in Table 14. The LD_{50} response for fenvalerate varied 5 fold from 0.004 - 0.02 µg / larva, the slope values ranged from 1.5 to 5.6. Prior to December 1983 the slope values were high, but some later collections exhibited lower slope values below 2.0. this suggests some response heterogeneity in *H.punctigera*. For deltamethrin the LD_{50} varied 3 fold from 0.002 - 0.006 µg / larva and there was considerable variation in the slope values (0.6 - 3.1). From December 1983 slope values less than 2.0 were found in 70% of *H.punctigera* collections tested.These data indicates a high degree of heterogeneity in these cultures.

Discussion

We consider that the data obtained for DDT, endosulfan and methyl parathion which showed high slopes and little variation of responses to be representative of susceptible *H.punctigera*. This can be used as baseline data, against which future changes can be measured. For the pyrethroids, our data showed that deltamethrin was intrinsically more toxic to *H.punctigera* than fenvalerate. Most *H.punctigera* appeared susceptible to fenvalerate, but it is likley that there was incipient resistance in a small percentage of the collections (16%). However, there were stronger indications of incipient deltamethrin resistance in a large proportion of the *H.punctigera* populations collected. Fenvalerate and deltamethrin are both α - cyano pyrethroids and it is not clear why the potential for resistance was greater for deltamethrin.

Pyrethroids have been used to control *Helicoverpa* spp in Australia since the first registations in the late 1970's. *H.armigera* resistance ledto pyrethroid field failures in late 1982 (Gunning *et al*, 1984). However, pyrethroids have achieved and still do achieve good control of *H.punctigera*. This data indicates that some *H.punctigera* in the field have been tolerant to fenvalerate and deltamethrin for some years.

The development of resistance is influenced by a combination of genetic, ecological, behavioural and agronomic factors. In Australia, both *H.punctigera* and *H.armigera* have been subject to intense insecticide selection, especially on cotton. Both species are highly polphagous). *H.punctigera* is extremely mobile and occurs on more uncultivated native and introduced host plants than *H.armigera*. These factors, combined with a more extensive geographic distribution may have allowed *H.punctigera* to maintain adequate populations in unsprayed refugia to dilute selection occurring in sprayed crops. *H.armigera* has lower numbers of uncultivated hosts and is mainly found in cropping areas. This means that a higher proportion of the population is exposed to selection on a regular basis . The findings of Forrester (unpublished data) and Gunning and Easton (1989) that pyrethroid resistance frequency in *H.armigera* populations is high and increasing, gives some validity to this hypothesis.

D.A. Murray has found that *H.punctigera* has a complex diapause strategy which could lessen the potential for insecticide selections. In subtropical and temperate Australia, *H.punctigera* may undergo winter, spring and summer diapause. This diapause behaviour, as well as conferring extra ability to cope with variable seasonal conditions may mean that *H.punctigera* has many sub-populations, some of which would escape insecticide selection.

Endosulfan and pyrethroids are key components of the *Helicoverpa* resistance management strategy.Our data shows that while *H.punctigera* are endosulfan susceptible there was considerable variation in the response of *H.punctigera* populations to pyrethroids. Although *H.punctigera* have the physiological and biochemical ability to develop pyrethroid resistance they have yet to develop field resistance to any chemical. These data suggest that for *H.punctigera*, resistance is not an inevitable consequence of pesticide use and that ecological factors may be as important as pesticide selection in influencing the development of resistance in *Helicoverpa* spp.

Table 13 Response of Australian *Helicoverpa punctigera* to insecticides. Insecticides were topically applied to 3rd instar larvae (30 - 40 mg) of the first labotatory generation.

Insecticide	**Site**	**Date**	**Crop**	**LD 50** µg/larva	**Fiducial limits**	**Slope**
DDT	Wee Waa,NSW	12/74	lucerne	2.0	1.6 - 2.7	2.0
	Wee Waa	1/75	cotton	2.8	1.4 - 4.3	2.2
	Wee Waa	1/75	cotton	2.2	1.8 - 2.9	2.2
	Moree,NSW	1/75	cotton	1.7	1.5 - 2.1	5.6
	Narrabri,NSW	1/75	cotton	1.8	1.2 - 2.8	2.3
	Wee Waa	3/75	cotton	2.0	1.5 - 2.7	2.3
	Moore Ck.,NSW	3/75	tobacco	1.7	1.4 - 2.1	3.8
	Narrabri	2/79	cotton	1.5	0.9 - 2.4	2.7
endosulfan	Wee Waa	1/75	cotton	1.4	1.2 - 1.6	2.7
	Breeza,NSW	11/84	light trap	1.0	0.8 - 1.2	2.5
	Tamworth	10/85	light trap	0.6	0.5 - 0.8	2.9
	Mareeba,Q	11/85	tobacco	1.1	0.8 - 1.3	3.5
	Belatta,NSW	12/85	sunflower	1.3	0.9 - 1.7	3.3
	Adelaide,SA	1/86	peas	1.4	1.3 - 2.0	2.6
	Spring Ridge,NSW	1/86	sunflower	1.7	1.3 - 2.0	2.4
	Boggabri,NSW	1/86	sunflower	1.3	0.8 - 2.0	2.2
	Kingower,Q	2/86	pigeon peas	0.7	0.6 - 0.8	5.6
	Bourke,NSW	2/86	cotton	1.0	0.8 - 1.2	3.5
	Tenterfield.NSW	5/86	sunflower	0.9	0.7 - 1.3	3.0
	Emerald,Q	5/86	cotton	1.2	0.7 - 1.7	2.2
	Breeza	11/86	light trap	0.8	0.6 - 1.3	2.7
	Tamworth	11/86	light trap	0.5	0.2 - 0.7	2.4
	Tamworth	12/86	light trap	0.9	0.6 - 1.2	2.6
	Mareeba	12/86	tobacco	1.2	1.0 - 1.4	4.3
	Moree	12/86	sunflower	0.6	0.4 - 0.8	3.2
	Kununurra,WA	7/87	sunflower	0.5	0.4 - 0.7	3.2
carbaryl	Wee Waa	12/74	cotton	6.2	4.2 - 9.1	1.6
	Wee Waa	1/75	cotton	3.0	2.0 - 4.2	1.4
methomyl	Emerald	12/86	cotton	0.02	0.01 - 0.03	2.2
	Breeza	5/88		0.01	0.007 - 0.014	2.5
methyl parathion	Wee Waa	1/75	cotton	0.22	0.18 - 0.27	3.6
	Tamworth,NSW	12/80	light trap	0.21	0.18 - 0.25	3.8
	Narrabri	2/81	cotton	0.14	0.12 - 0.17	4.0

Table 14 Response of Australian *Helicoverpa punctigera* to pyrethroid insecticides. Fenvalerate and deltamethrin were topically applied to 3rd instar (30 - 40 mg) larvae.

Insecticide	Site	Date	crop	LD 50 µg/larva	Fiducial limits	Slope
deltamethrin						
	Warren,NSW	5/83	cotton	0.005	0.004 - 0.006	3.1
	Tamworth,NSW	10/83	light trap	0.002	0.001 - 0.003	3.0
	Breeza,NSW	12/83	light trap	0.006	0.004 - 0.009	1.4
	Sydney,NSW	12/83	light trap	0.004	0.001 - 0.008	1.2
	Tamworth	12/83	light trap	0.002	0.0006 - 0.02	0.6
	Breeza	12/83	light trap	0.006	0.004 - 0.006	2.1
	Lorne,VIC	1/84	peas	0.003	0.002 - 0.005	2.1
	Tamworth	9/84	light trap	0.004	0.002 - 0.006	1.3
	Tamworth	10/84	light trap	0.005	0.004 - 0.007	1.8
	Tamworth	10/85	light trap	0.004	0.003 - 0.006	1.6
	Breeza	11/84	light trap	0.003	0.002 - 0.005	1.6
	Kingaroy,Q	3/86	light trap	0.004	0.002 - 0.007	1.4
	Bourke,NSW	3/86	cotton	0.004	0.003 - 0.005	2.8
fenvalerate						
	Narrabri,NSW	1/77	cotton	0.009	0.007 - 0.015	2.3
	Wee Waa,NSW	5/77	cotton	0.012	0.009 - 0.015	2.5
	Warren	2/78	cotton	0.007	0.006 - 0.009	3.4
	Narrabri	2/79	cotton	0.012	0.008 - 0.013	4.0
	Sydney,NSW	11/83	light trap	0.006	0.004 - 0.009	2.9
	Tamworth	12/83	light trap	0.007	0.006 - 0.009	2.3
	Capella,Q	12/83	cotton	0.012	0.009 - 0.016	2.3
	Breeza	12/83	light trap	0.006	0.004 - 0.009	1.6
	Breeza	12/83	light trap	0.006	0.004 - 0.008	1.7
	Warren	1/84	cotton	0.006	0.005 - 0.007	4.7
	Lorne	1/84	peas	0.010	0.008 - 0.013	3.2
	Tamworth	3/84	pigeon pea	0.015	0.008 - 0.022	1.6
	Tamworth	9/84	light trap	0.006	0.005 - 0.007	3.4
	Breeza	10/84	light trap	0.004	0.003 - 0.005	2.4
	Tamworth	10/84	light trap	0.007	0.006 - 0.008	3.5
	Tamworth	10/84	light trap	0.006	0.005 - 0.007	3.4
	Tamworth	10/84	light trap	0.007	0.006 - 0.008	2.1
	Narrabri	2/85	cotton	0.008	0.005 - 0.010	2.2
	St.George,Q	3/85	cotton	0.008	0.006 - 0.010	2.9
	Emerald,Q	10/85	sunflower	0.007	0.005 - 0.009	2.7
	Bellata,NSW	12/85	sunflower	0.007	0.006 - 0.008	5.6
	Moree,NSW	1/86	sunflower	0.008	0.006 - 0.010	2.7
	Adelaide,SA	1/86	peas	0.008	0.006 - 0.010	2.3
	Bourke	1/86	cotton	0.013	0.009 - 0.018	2.6
	Bathurst,NSW	1/86	prickly pear	0.005	0.004 - 0.006	3.6
	Spring Ridge,NSW	1/86	sunflower	0.009	0.007 - 0.011	3.3
	Kingaroy	1/86	pigeon pea	0.007	0.006 - 0.009	4.3
	Tamworth	2/86	light trap	0.006	0.004 - 0.008	3.8
	Bellata	5/86	sunflower	0.013	0.009 - 0.018	3.0
	Emerald	5/86	sunflower	0.013	0.010 - 0.018	2.7

Tenterfield	5/86	sunflower	0.017	0.013 - 0.022	2.1
Tamworth	9/86	light trap	0.014	0.007 - 0.020	1.8
Breeza	9/86	light trap	0.018	0.011 - 0.025	1.8
Emerald	10/86	sunflower	0.020	0.014 - 0.025	3.8
Tamworth	11/86	light trap	0.015	0.011 - 0.020	1.6
Moree	11/80	sunflower	0.013	0.011 - 0.016	2.9
Tamworth	11/86	light trap	0.016	0.013 - 0.019	4.0
Emerald	11/86	cotton	0.010	0.007 - 0.014	1.9
Tamworth	12/86	light trap	0.011	0.009 - 0.014	3.1
Spring Ridge	12/86	sunflower	0.014	0.010 - 0.018	3.6
Mullalley,NSW	12/86	sunflower	0.009	0.006 - 0.013	1.6
Mareeba,Q	12/86	tobacco	0.014	0.011 - 0.018	2.8
Pine Ridge,NSW	12/86	sunflower	0.006	0.003 - 0.009	2.8
Moree	12/86	sunflower	0.008	0.005 - 0.012	3.1
Pallamallawa,NSW	12/86	sunflower	0.010	0.008 - 0.013	2.2
Moree	12/86	sunflower	0.008	0.006 - 0.010	2.4
Moree	12/86	sunflower	0.011	0.004 - 0.019	1.8
Moree	12/86	sunflower	0.007	0.003 - 0.020	1.6
Narrabri	12/86	cotton	0.008	0.006 - 0.011	2.7
Breeza	1/87	light trap	0.012	0.008 - 0.019	1.5
Kununurra,WA	7/87	sunflower	0.005	0.003 - 0.007	2.7
Bowen	10/87	tomatoes	0.005	0.002 - 0.008	3.1
Tamworth	9/88	light trap	0.009	0.006 - 0.014	3.2
Kununurra	1/89	soybeans	0.013	0.010 - 0.016	4.5

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