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**Field of Research:** Entomology **Field code:** 3.1


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# Management of pyrethroid and endosulfan resistance in *Heliothis (=Helicoverpa) armigera* (Hübner) in Australia.

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# PROLOGUE

In January 1985, Professor C.E. Taylor addressed the Linnean Society of London making the comment "I can think of few problems in evolutionary biology that are more important than controlling resistance, a problem that is serious enough now, and certain to become more so". His prophetic comment remains as pertinent now as it did then for, despite significant advances in our knowledge of the genetics, physiology and biochemistry of resistance, little progress has been achieved in formulating practical countermeasures against the inexorable march of resistance. This study is an attempt to address this problem.

# CHAPTER 1 - The Australian Insecticide Resistance Management Strategy


## Abstract

*In response to field pyrethroid failures against* Heliothis armigera *(Hübner) in early 1983, an Insecticide Resistance Management (IRM) Strategy was introduced for insect control in summer crops in eastern Australia. The aims of this Strategy were to contain the pyrethroid resistance problem, to prevent re-selection of historical endosulfan resistance (both curative IRM) and to avoid any future problems with organophosphate/carbamate resistance (preventative IRM). An alternation strategy was adopted which was based on the rotation of unrelated chemical groups on a per generation basis, along with a strong recommendation for the use of ovicidal mixtures. These chemical countermeasures were then integrated with other non-chemical control methods (biological and cultural) into a workable Integrated Pest Management Programme. The restrictions were applied to all* Heliothis armigera *susceptible crops (including cereals, oilseeds, grain legumes, tomatoes, tobacco and cotton) and even to other coincident pest species. Compliance with the voluntary strategy has been exceptional, right from its inception.*


## Introduction

In January 1983, pyrethroids failed to give satisfactory field control of *Heliothis* (=*Helicoverpa*) *armigera* (Hübner) at Emerald in central Queensland. Prior to that, as in the USA (Riley 1989), they had been "heralded as miracle insecticides" as they replaced the resistance prone and environmentally liable organochlorines, cyclodienes and organophosphates (Morton & Collins 1989). When they were introduced commercially in the late 1970's, they had many benefits over what was then available. They were very cost effective at extraordinarily low rates on a broad range of agricultural and public health pests, had no residue problems, were safe to mammals, had low environmental impact and were immobile in the soil (Elliott 1989). Indeed, they were regarded as the almost perfect insecticide (Leahey 1985). Infact, by 1986, their popularity was such that they accounted for around 25% of all insecticides used in agriculture and public health (Jackson 1989, Hirano 1989). They were particularly favoured in cotton because of their contact mode of action and good efficacy against previously resistant pests and by the mid 1980's accounted for 49% of the world cotton insecticides market (Watkinson 1989, Riley 1989). So when the breakdown at Emerald was clearly shown to be due to the development of resistance (Gunning et al. 1984), there was no disguising the concern of the Australian cotton industry in particular, but also the other field crop industries in which *H.armigera* was a key pest. Within 6 months of these reported field failures, a strategy aimed at containing the resistance problem, had been formulated and ratified for use in the following season, by all parties concerned (Forrester 1990).

## Background, Format & Aims of the Australian Strategy

Insecticide resistance has been a recurring problem for Australian summer crop, particularly cotton, growers. *Heliothis armigera* has developed resistance to virtually every insecticide group used against it, including the organochlorines, cyclodienes, organophosphates, carbamates and pyrethroids (Fig 1.1). Although prompted by the development of resistance to pyrethroids, the Australian Strategy does not just aim to manage pyrethroid resistance. Because of a predicted increased reliance on alternative insecticides with previous resistance histories (particularly endosulfan), it was decided from the outset that the aim should be to manage resistance to all the available chemical groups. These included the pyrethroids, endosulfan and the organophosphates/ carbamates. A different approach was used for each group, depending on the severity of the resistance risk and predicted selection pressure.

Pyrethroid and endosulfan resistance management was designed mainly on an alternation strategy based on rotation of chemical groups on a per generation basis. Pyrethroids (maximum of three) were recommended to be used for a 42 day period (Stage II window) during the middle of the season (Fig 1.2). This 42 day period corresponded to the minimum time required for the development of one generation of *H.armigera* in the field (Room 1983). Thus, pyrethroid selection pressure was restricted to 1 of the 4-5 generations per season. However, because of the tendency for growers to apply a pyrethroid late in the Stage II window and the residual nature of the pyrethroids, it was found that a 42 day pyrethroid window was selecting for more than one generation, particularly in hotter than average seasons. Thus, it was decided to reduce the 42 day window to 35 days from the 1989/90 season onwards. Endosulfan was recommended to be used in either Stage I or II but not in Stage III (cotton only). The retention of endosulfan for use on non-cotton crops in Stage III was based on its relatively low use in these crops and the lack of any registered alternatives. Thus, endosulfan selection pressure was restricted to 3 of the 4-5 generations per season. The restrictions on endosulfan were less severe than those for the pyrethroids as the resistance problem was not considered as acute. Endosulfan useage was also expected to be targetted principally early season in Stage I where *H.armigera* is much less of a problem. In addition to these restrictions there was a further recommendation to add an ovicide (principally methomyl and chlordimeform) to pyrethroids and/or endosulfan when egg pressure warranted. Thus larvicide/ovicide mixtures were commonly used as another method of resistance management. Mixtures of larvicides with larvicides from another chemical group, as suggested in the literature, were not attempted as no economically justifiable combinations could be found.

The approach to management of organophosphate and carbamate resistance was slightly different as there were no known previous resistance problems for any of the available organophosphate and carbamate insecticides (except for parathion). Also their use was predicted to be minimal (in comparison with the pyrethroids and endosulfan) so a less restrictive preventative approach was taken. Their use was not restricted to any Stage as it was predicted that their major use period would be in Stage III when both pyrethroids and endosulfan were restricted. It was

predicted that the cheaper cost effective insecticides would be used in Stage I (endosulfan) and Stage II (pyrethroids) and that the more expensive organophosphates and carbamates would be only used in these Stages as and when necessary (eg. for control of coincident mites and *Heliothis* spp.). Thus, it was considered that market forces would have confined their use to the preferred option anyway (i.e. mostly in Stage III), so that external regulation was considered unnecessary. This resulted in a preventative mosaic approach (similar to Byford et al. 1987) for the management of organophosphate and carbamate resistance, compared to the more restrictive curative rotation approach for pyrethroids and endosulfan.

Because of its polyphagous nature, it was recommended that all growers of *H.armigera* susceptible crops should adopt the Strategy and be subject to the same time constraint. Consequently the Strategy applies to all cereal, oilseed, grain legume, tomato and tobacco crops as well as to cotton, the main crop at risk (Forrester 1987). The restriction on pyrethroid use in *H.armigera* susceptible crops also applies to other insect pests such as sorghum midge (*Contarinia sorghicola*), armyworms (*Mythimna convecta*) and *Nysius* spp. bugs, which are often present with *H.armigera*. It was suggested that spraying of these pests with pyrethroids would also select inadvertently for resistance in *H.armigera* (see Chapter 6). Because of the multicrop nature of the Strategy, the timing of the pyrethroid window was designed to satisfy, as much as possible, the needs for insect control in each crop. Thus, in cotton, it was targetted to peak flowering/early boll set, a vulnerable period of the cotton growth cycle when the highly efficacious contact pyrethroids would be most appreciated. It was also designed to cover the peak sorghum flowering period so that the pyrethroids would be available to sorghum growers for midge control (another significant pyrethroid market).

Although the main emphasis of the Strategy as outlined so far, would seem to be on chemical countermeasures, this is far from the case. The Insecticide Resistance Management (IRM) Strategy was specifically designed to fit into a broader Integrated Pest Management (IPM) programme as well (Forrester 1990a). For example, pyrethroids were avoided early season, (replaced by the "softer" insecticides such as endosulfan, thiodicarb and *Bacillus thuringiensis*) so that there would be minimal disruption to the early season beneficial parasites and predators and also to avoid the potential flaring of secondary pests such as mites, aphids and whitefly. There were also a number of key Strategy guidelines which recommended additional non-chemical countermeasures to reduce selection pressure (Forrester 1990b). For example:-

- Grow early maturing crops to avoid dominant *H.armigera* populations late season
- Avoid growing certain alternative host crops (especially early maize and sunflowers) near cotton, as they serve as early season nursery crops for resistant *H.armigera*.
- Avoid consecutive sprays of pyrethroids where *H.armigera* are emerging from neighbouring early season alternative host crops, as resistance levels will be exacerbated by selection of moths before mating (see Chapter 7).

• Sample over-wintering pupae under cotton stubble and cultivate if they exceed threshold (Fitt & Forrester 1987)

• Target pyrethroids to egg hatch, to avoid selection of older established larvae (Daly et al. 1988).

• Scout crops frequently and thoroughly and spray on threshold. This can minimise the need for sprays and ensure their maximum effectiveness through optimum timing (especially important for the shorter residual organophosphates).

• Utilise host plant resistance wherever possible (eg. okra leaf varieties offer some degree of control, particularly for mites)

• If a pyrethroid is used to control sorghum midge, do not follow up with a pyrethroid for heliothis control, as the midge spray will have already selected for pyrethroid resistant *H.armigera* (see Chapter 6).

This integrated approach was designed to spread the selection pressure over a number of mortality factors so that *H.armigera* did not have the opportunity to concentrate its efforts to develop resistance to any one control measure.

## Discussion

Insecticide use surveys (see Appendix 3) indicated universal adoption of the Strategy right from its inception. This was rather pleasing as it was, and still is, only a voluntary Strategy. However, the high compliance rate was not altogether a surprise, as the Australian cotton industry was well aware of the economic consequences of uncontrolled resistance. They had experience of DDT and DDT/toxaphene resistance in the early 70's, particularly in the Ord (Hearn 1975) and endosulfan resistance in the mid 70's. They understood that if countermeasures were not taken, that their industry was at risk to reduced profitability at first and ultimately, to complete abandonment, as had happened on a number of previous occasions throughout the world (eg. Bottrell & Adkisson 1977, Matthews 1989, Vaughan & Leon 1976, Hearn 1975, Dover & Croft 1984, Nat. Acad. Press 1986). The insecticide use patterns proved to be as anticipated with Stage I sprays being mainly endosulfan, Stage II mainly pyrethroids and Stage III organophosphates (Appendix 3). Thus the basis of the Strategy, rotation of unrelated chemical groups (with 3 different sites of action, Hammock & Soderlund 1986), has been adopted in practice.

In their tome on Resistance Management, the National Academy of Science (1986) suggested that "although the theory and observations of academic population biology have been used to explain past resistance episodes, at this juncture (1984), there have not been significant pesticide use programs developed and implemented from considerations of the principles of population biology". This is not surprising due to the necessarily hurried approach to solving the pressing problems of reactive curative resistance management. However, a concerted effort was made to incorporate as much knowledge of poplulation biology in the Australian Strategy as was possible, given the lack of specific models at the time. For example, May & Dobson's (1986) concept of a

population usually requiring longer to recover susceptibility than it did to acquire resistance, was accounted for from the start (eg. a selection interval of 1 generation was allowed, followed by a regression interval of 3-4 generations to allow for typically weaker back selection than insecticide selection pressure). The selection interval was also based on a logical population biology criterion (i.e. minimum generation time) while host range and phenology, interacting pest biologies, moth dispersal capacity as well as political, social and agronomic constraints, were all taken into account. It is hoped that subsequent reviews of IRM strategies will recognise these genuine efforts to legitimise the science of practical IRM.

Various IRM Strategies have been adopted throughout the world (reviewed by Sawicki & Denholm 1989) however the Australian Strategy remains the world's first attempt at nationwide curative resistance management. As it is generally agreed that it is undoubtedly easier to suggest strategies for delaying resistance (= preventative IRM) than to recommend countermeasures once resistance has appeared (= curative IRM) (Wood & Mani 1981), it was decided to concentrate on the most difficult and pressing component of the Australian Strategy (that is the problem of curative management of pyrethroid and endosulfan resistance). These are the two most widely used insecticide groups used in Australian summer field crops, accounting for over 80% of insecticide use against *Heliothis* spp. So their loss to resistance would have a major economic impact, particularly in cotton. The next two chapters evaluate the effectiveness of the Australian IRM Strategy in managing resistance to these two key insecticides.

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## Historical Insecticide Use & Resistance Spectrum of *Heliothis armigera* in Australia

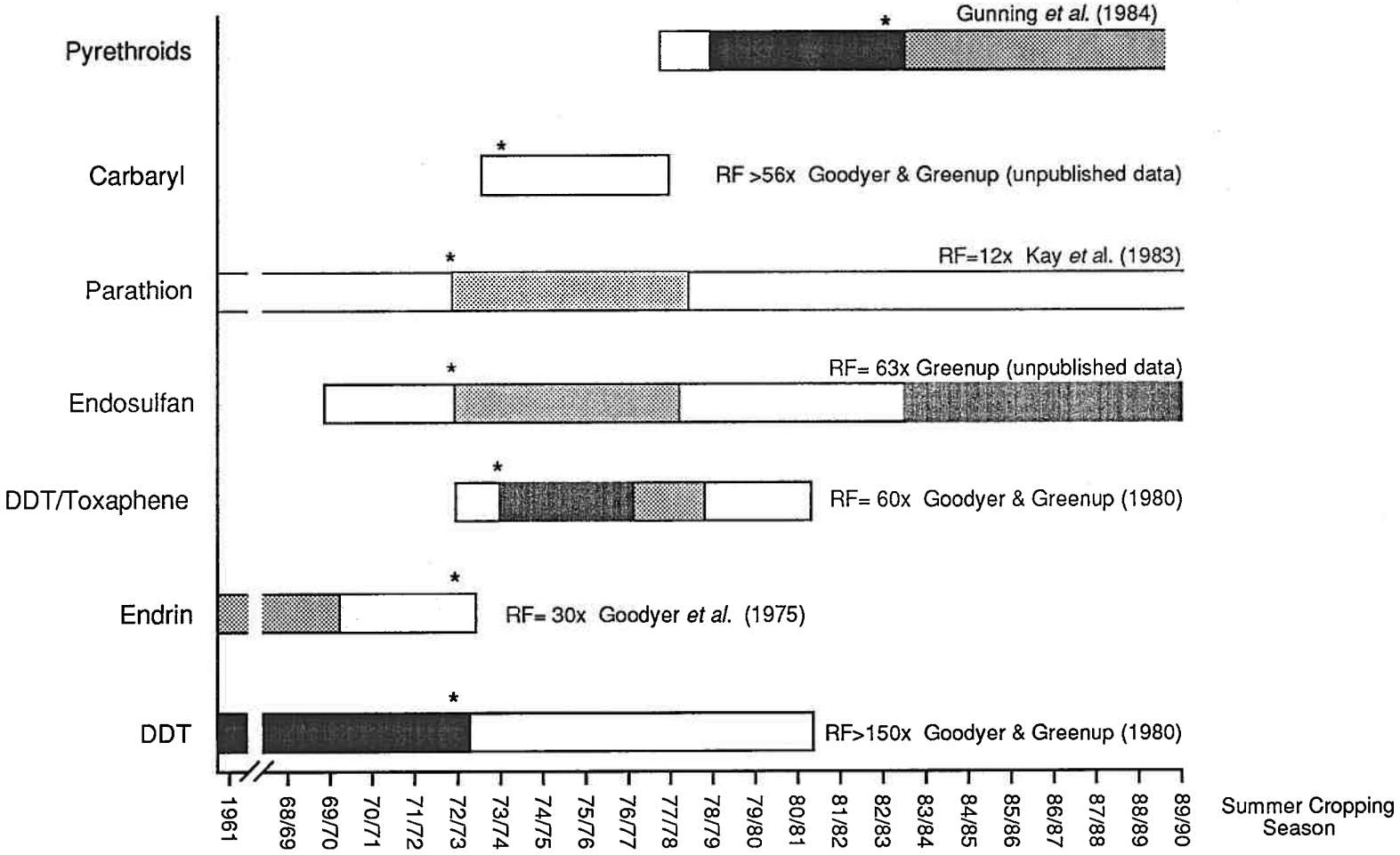


**Fig 1.1** Historical sequence of insecticide use in Australian cotton (first significant commercial crop 1961) and the development of resistance in the recidivist cotton & field crop pest *Heliothis armigera*. Horizontal bars indicate the duration & intensity of use for each insecticide ( low ▭ , moderate ▒ , high ■ ). * indicate the first records of field resistance. RF's indicate the maximum recorded resistance factors.

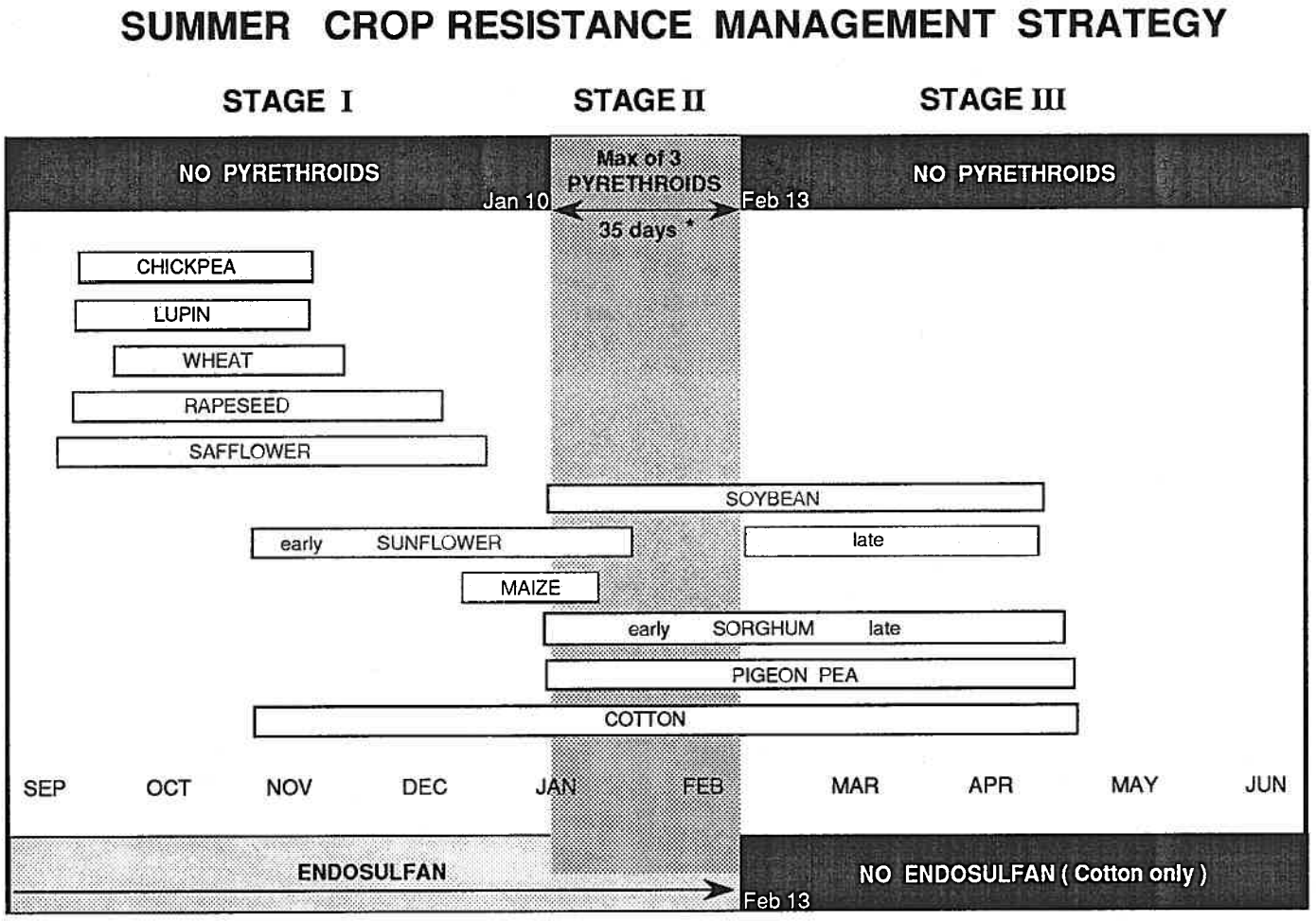


**Fig 1.2** Summer Crop Resistance Management Strategy for northern New South Wales and southern Queensland (the Emerald Irrigation Area of central Queensland has an earlier Stage II window, beginning Jan 1 and finishing Feb 3). Crop intervals indicate the periods when control of *Heliothis* spp. (or other contemporaneous pests such as sorghum midge (*Contarinia sorghicola* ), armyworms (*Mythimna convecta* ) or *Nysius* spp.) may be required in those crops. * 1983/84 to 1988/89 Stage II window 42 days duration (Jan 10-Feb 20); 1989/90 onwards, reduced to 35 days.

# CHAPTER 2 - Evaluation of the Impact of the Strategy on Pyrethroid and Endosulfan Resistance : Discriminating Dose Studies


## Abstract

*The monitoring technique employed in this study (discriminating dose screening of larvae reared from field collected eggs) proved extremely successful in documenting the impact of the strategy on both pyrethroid and endosulfan resistance, without the problems of alternative techniques. Because of the sensitivity of this technique, Strategy users have been able to verify the anticipated impact of the Strategy, identify problems, adjust their management practices accordingly and assess the effectiveness of these initiatives. This has been instrumental in maintaining the Strategy's excellent compliance rate.*

*Pyrethroids selected for resistance in both moths and larvae, resulting in increases in resistance within the Stage 2 window and the early Stage 3 period, respectively. These two peaks effectively merged into one large peak whilever the pyrethroid use period remained at 42 days. However, the initiative to reduce the pyrethroid window to 35 days, separated the two peaks and proved to be a successful delaying tactic. The two main factors influencing pyrethroid resistance were dilution by susceptibles immigrating from the refugia, followed by pyrethroid selection pressure. However, as the refugia became increasingly contaminated, its effectiveness as a source of susceptibles for dilution declined, resulting in gradually increasing pyrethroid resistance levels in all areas over time. Adult selection was more important in the mixed cropping Emerald study area because of premating selection. This, alongwith the higher* Heliothis armigera *pressure at Emerald, probably offset any potential benefit of the longer cropping season at this site. Inadequate cultivation of overwintering pupae correlated well with low price forecasts in the economically sensitive cotton industry and resulted in the carryover of large numbers of resistant pupae. As a result, cultural control of overwintering pupae has become a major component of the integrated Australian Resistance Management Strategy. The Strategy has not overcome the pyrethroid resistance problem but has proven to be a successful delaying tactic in buying time and extending the useful life of the pyrethroids.*

*However, the Strategy has been much more successful in managing endosulfan resistance and some possible reasons for this are discussed:- effectively lower selection pressure, fitness deficit, fewer life stages selected or less genetic dominance. However, it was not possible from this study to determine the relative importance of these factors or their interactions.*


## Introduction

The need to monitor resistance has been widely recognised for some time (Dennehy 1987, Dover & Croft 1986, Georghiou & Taylor 1986, National Academy Press 1986, Hammock & Soderlund 1986, Cook 1981). Indeed, Dennehy (1987) considered "monitoring methodology the vehicle needed to make most Resistance Management Strategies implementable and verifiable". This need

was also recognised very early on in the planning of this Strategy and considerable effort was given to design a monitoring system which could simply and accurately indicate the impact of the Strategy on pyrethroid and endosulfan resistance. A technique based on discriminating dose screening of larvae reared from field collected eggs was adopted, as it was considered simple, accurate and efficient (see Appendix 2 and references therein). The classical resistance monitoring technique using full bioassay lines on laboratory reared F1 progeny of field material, was also evaluated for comparative purposes (see Chapter 3).

Previous resistance studies have evaluated discriminating doses on field material (eg. Georghiou & Taylor 1976, Pree & Wagner 1987) or on F1 progeny of field material (eg. Denholm et al. 1983, Roulston et al. 1981). Some studies have even attempted to correlate operational and biological factors with changes in resistance (eg. Wolfe & Barrett 1986, Georghiou et al. 1973). However, none were designed to specifically monitor the impact of a Resistance Management Strategy on a continuous and long term basis. Nor were they all sensitive enough or backed by sufficient detailed ecological and operational data, to allow an accurate assessment of the relative importance of these factors. Wood and Bishop (1981) recognised that "management of resistance is an aspect of applied ecological genetics". This study, designed with that comment in mind, aims to demonstrate that Insecticide Resistance Management is founded on sound ecological principles.

## Methods & Materials

### *Sampling Areas* (Fig 2.1)

Three ecologically contrasting areas were chosen for this study. The first site was the Namoi and Gwydir river valleys of northern New South Wales (downstream of Narrabri and Moree, respectively) which are essentially a large monoculture of irrigated cotton, averaging 50-60,000 hectares of cotton per season (Fig A3.1). The second site chosen was the Emerald Irrigation Area of central Queensland which is the most northerly cotton growing area in Australia, centred on the Tropic of Capricorn. This is a smaller mixed cropping area capable of growing up to 12,000 irrigated hectares of various crops, but mainly cotton (Table 2.3). This was also the site of the most serious pyrethroid field failures in the 1982/83 season. Being further north, Emerald has a generally milder and therefore longer summer cropping season than the Namoi/Gwydir. For example, while *Heliothis armigera* has only 4-5 generations per season in the Namoi/Gwydir, it can have up to 6-7 at Emerald. Therefore sampling at Emerald usually started earlier and finished later (September-May) than in the Namoi/Gwydir (November-April). While the first two sites chosen were intensively sprayed cotton areas, the third was an essentially unsprayed refugia area of dryland (raingrown) alternative host crops (mainly maize, sorghum and sunflowers) and the scrophulariaceous weed host *Verbascum virgatum*. This smaller unsprayed area, centred just west of Inverell in northern New South Wales, is within 50-100 kms of the intensively sprayed Namoi/Gwydir study area.

The Namoi/Gwydir site was sampled from the very first season (1983/84) following the introduction of the Resistance Management Strategy but sampling at Emerald was delayed for two

seasons until 1985/86 because of difficulties in organising an intensive monitoring programme at this relatively remote site (some 1,000 kms from the central Narrabri laboratory). Because of the importance of the refugia as a source of susceptibles for dilution of resistance, a sample of the refugia (the Inverell area) was incorporated into the programme from 1987/88 onwards.

The same sampling areas have been used each season and collection sites within each study area are chosen randomly. A conscious effort was made to spread the collection sites evenly throughout each study area and to avoid concentrating on resistance hot spots or including samples from outside the originally chosen areas as this can easily bias results (eg. Plapp et al. 1990).

### *Sampling Procedure*

Each property was considered as a basic sampling unit. Eggs were collected at random from as many fields as possible for each property (ideally up to 300-400 eggs/property). A conscious effort was made not to collect eggs from within just a small area in each field in order to avoid the possibility of collecting eggs laid by the same moth. Hopefully, these efforts achieved the ideal situation of each collected egg having been laid by a different female. Eggs laid on the leaves, squares, buds, flowers, stems or silks of the various host plants, were collected into muslin bags (eggs left attached to plant material) and kept cool during transport back to the laboratory. Eggs were collected each working day throughout the growing season wherever possible, except at Inverell where samples were taken either once a week or once a fortnight.

### *Sample Processing*

On receipt in the laboratory, eggs were removed off the plant material with a fine paintbrush moistened in 0.1% sodium hypochlorite, placed on artificial diet in tissue culture trays (one egg per well) and sealed with a semi-permeable plastic wrap to prevent escape of neonates (See Appendix 1 for details of diet, rearing methods etc). Every effort was made to transfer the eggs before hatching, to avoid neonates being possibly exposed to any residual spray deposits. Eggs from the Namoi/Gwydir and Inverell study areas were reared immediately at $25^{o}C \pm 2^{o}C$ but samples from the remote Emerald site were held at $12^{o}C$ and despatched at weekly intervals in insulated transportable coolers, to the central testing laboratory at Narrabri. Samples from Emerald arrived usually in 2-3 days but as neonates had access to artificial diet, hatching in transit was not a problem.

Hatched larvae were speciated at the 2nd or 3rd instar to either *Heliothis armigera* or the susceptible sibling species *Heliothis punctigera*. The *H. punctigera* larvae were either discarded or screened as described in Appendix 4. The *H. armigera* larvae were then reared to 30-40 mg and screened as either 3rd or 4th instars with the relevant fenvalerate discriminating dose (see Appendix 2) to determine pyrethroid resistance. It was found necessary to check the fast growing larvae twice a day (only once per day on weekends and holidays) to maximise the yield of suitably sized testing larvae. Late 3rd instar moulting larvae were avoided and were held overnight at $18^{o}C$ and tested the next day as either 30-40 mg or 40-60 mg 4th instars with the appropriate fenvalerate discriminating dose (either

0.2 or 0.5 ug/larvae, respectively, see Appendix 2). Most larvae (approx 90%) were tested at the lower weight range. The development of this 'twin' discriminating dose technique proved critical to the economic success of this labour intensive programme as very little of the valuable field material missed being tested at either weight range. This was especially important during Stage 1 when *H. armigera* numbers were at their lowest.

In 1986/87 season, a second insecticide (endosulfan) was incorporated into the field screens. Starting with that season, *Heliothis armigera* larvae from each sample were split equally and randomly into two subsamples and tested with either the fenvalerate discriminating dose (as previously) or the endosulfan discriminating dose (10 ug per 30-40 mg larva, see Appendix 2). As there was no endosulfan discriminating dose determined for the 40-60 mg weight range, larvae from the endosulfan subsamples which grew through the 30-40 mg testing weight range, were transferred to the fenvalerate subsample and tested with the higher fenvalerate discriminating dose.

## *Sample Analysis*

Resistance levels were expressed as the percentage of larvae surviving the discriminating dose. Each property was considered as a separate sample except where *H. armigera* numbers were considered too low for satisfactory analysis (less than 20 larvae per fenvalerate or endosulfan screen). In these cases, samples from different properties were combined (on the basis of spatial and temporal similarity), so that a minimum sample size of 20-25 larvae was obtained. The samples were then either pooled into collecting weeks and graphed using between site binomial standard error estimates (Figs 2.4,5 & 6, Table 2.1) or into collecting Stages (1,2 or 3) and tabled or graphed using standard errors of the mean (Table 2.2, Fig 2.7). Comparisons were also made between the weekly pooled and between site binomial standard errors (Table 2.1), as suggested in Sawicki et al. (1989). The significance of the indices of Total Season Selection Pressure (increase in pyrethroid resistance between Stages 1 and 3, Table 2.5), Inter-Season Decline (decrease in pyrethroid resistance between previous Stage 3 and the following Stage 1, Table 2.4) and Adult Selection (increase in pyrethroid resistance between Stages 1 and 2, Table 2.3), were all made using unpaired 't' tests on combined Stage means.

## *Rainfall & Crop Surveys*

Rainfall records for 45 sites spread throughout the New South Wales portion of the eastern Australia summer rainfall cropping belt (Fig 2.1), were obtained from the Bureau of Meteorology. The average monthly summer rainfall (December to February) was expressed as a percentage of the long term average (Fig 2.8). This time period was chosen as rainfall in these months would impact on the growth of sorghum, the major alternate host flowering during Stage 2 in this region. In addition, sorghum production records for the same area were obtained from the Australian Bureau of Statistics and NSW Agriculture & Fisheries (Fig 2.8). This allowed a better assessment for the Stage 3 dilution potential of the surrounding refugia area than just rainfall data or sorghum area alone, as

sorghum production takes into account both the quantity (area) and quality (amount of rain) of this important dryland (raingrown) alternate host.

### *Winter Cultivation Surveys*

After recognising the critical importance of the overwintering population in carrying over resistance from one season to the next, an annual winter cultivation survey was instigated from 1987 onwards (Table 2.6). The survey was carried out in late September/early October, just prior to sowing and moth emergence from diapause, to allow the maximum available period for stubble management decisions to be undertaken. Cultivation practices were classed as either ineffective (little or no soil disturbance) or effective (stalks disturbed) in killing overwintering pupae of *Heliothis armigera* (Table 2.6).

## Results

### *Species composition* (Figs 2.2 & 3)

Namoi/Gwydir *H. armigera* Stage1 levels were generally quite low (0-20%), except for a variable peak in the early December period. This peak may be the first *H. armigera* generation on cotton but in fact, it is the second *H. armigera* generation of the season in the Namoi/Gwydir. Stage 1 *H. armigera* levels at Emerald were quite variable from season to season, probably reflecting changing cropping patterns in response to variable rainfall. However, it is quite clear that Stage 1 *H. armigera* pressure at Emerald is significantly higher than in the Namoi/Gwydir. Stage 2 *H. armigera* levels, both in the Namoi/Gwydir and Emerald, were generally higher than in Stage 1, particularly towards the end of this period. The changeover to late season *H. armigera* dominance was generally complete in State 3 in both areas, except for a few periods of *H. punctigera* pressure in March.

### *Pyrethroid resistance - Namoi/Gwydir*

Each season showed a similar pattern with slight but significant increases between Stages 1 and 2 (Tables 2.2 & 3) and sharp peaks in Stage 3 (Fig 2.4), except in the 1989/90 shortened pyrethroid window season. Normally, resistance peaked in the first few weeks of Stage 3 (early March) but in the 1989/90 season, it in fact dropped during this period, peaking only in late March. This resulted in a 'twin peak' quite distinct from previous seasons with longer pyrethroid windows (Fig 2.4). The apparent twin peak of 1986/87 was quite different from the 1989/90 season in that the trough occurred in the last 2 weeks of the Stage 2 pyrethroid window, not in Stage 3 (Figs 2.4 & 9). This was the only season when pyrethroid resistance declined within the pyrethroid window and coincided with a swing away from pyrethroids during the latter half of the Stage 2 window in this season (Fig 2.9), because of serious resistance problems.

Stage 1 levels returned to quite low levels (less than 10%) for the first 3 seasons but showed an alarmingly high increase early in the 1986/87 season (Fig 2.7). This coincided with high survival of the highly resistant overwintering pupae (44.5% average resistance for the previous Stage 3, Table 2.2)

which were not destroyed by cultivation during the 1986 winter because of record low prices on the New York Cotton Futures (Table 2.6, Fig 2.10). In fact, the Index of Inter-Season Decline for this winter, was the lowest recorded during the study (Table 2.4). Growers responded to these gloomy price forecasts by cutting back their cotton areas for the following season, sowing only their best fallow fields and leaving cotton stubble either uncultivated or sown to alternative winter crops instead of working them up for following cotton crops (Table 2.6). After a return to more stable prices, stubble management and crop rotation decisions also returned to normal (Table 2.6). The following Stage 1 (1987/88 season) also indicated a return to normality with a significant decline in resistance but not quite to the low levels of the first 3 years of the Strategy (Fig 2.7, Table 2.2). In fact, overall, there has been a clear and steady increase in resistance levels, in all three Stages over time (Fig 2.7).

### *Pyrethroid resistance - Emerald*

Resistance patterns were remarkably similar to those found in the Namoi/Gwydir with some important differences. For example, the increases in resistance between Stages 1 and 2, tended to be higher than in the Namoi/Gwydir, despite often lower selection pressure (Table 2.3). This Index of Adult Selection correlated well with the area of maize sown at Emerald, being highest when maize was a significant alternate crop in the Irrigation Area (Table 2.3). There were also some differences due to the longer season at Emerald, where Stages 1 and 3 each span multiple (2-3) generations, instead of a single generation as in the Namoi/Gwydir. This resulted in secondary lower resistance peaks late in Stage 3 (around April), about a generation after the first Stage 3 peaks in late February (Fig 2.5). The longer Stage 3 at Emerald also allowed more time for dilution and Stage 3 resistance levels at Emerald were significantly lower than in the Namoi/Gwydir, despite similar Stage 2 levels (Fig 2.7, Table 2.2). Despite these longer regression intervals (see Chapter 1) at Emerald, the trend to steadily increasing resistance in all three Stages over time, noted in the Namoi/Gwydir, was also clearly evident at Emerald (Fig 2.7). Also, the Stage 3 second generation peak in 1989/90 season, was just as high (approx 60%) as the earlier February peak, whereas in previous seasons, these later peaks had been significantly lower (Fig 2.5). The lack of a Stage 3 peak in 1985/86 was due to the very low pyrethroid selection pressure in that season because of the late timing of the pyrethroid window (see Appendix 3).

The Stage 1 resistance levels for the first two monitoring seasons at Emerald, were similar to the early Stage 1 figures for the Namoi/Gwydir (Table 2.2). The 1985/86 Stage 1 levels are particularly interesting as they indicate that pyrethroid resistance levels, after two seasons of non use at Emerald (see Appendix 3), had declined to levels no lower than where pyrethroids had been used each season (Table 2.2). The 1987/88 Stage 1 data are also interesting as they clearly show a spring to early summer decline in pyrethroid resistance during the multiple generation Stage 1 period (Fig 2.5). However, recent Stage 1 patterns at Emerald, have been quite variable and difficult to interpret (Fig 2.5).

The twin Stage 2/Stage 3 peak noted for the 1989/90 shortened pyrethroid window season in the Namoi/Gwydir (Fig 2.4), was not so clearly evident at Emerald (Fig 2.5), probably because of lower pyrethroid use at this site.

### *Pyrethroid resistance - Inverell Refugia*

Pyrethroid resistance levels at the start of the season (Stage 1) have increased to similar levels as those found in the nearby sprayed Namoi/Gwydir cotton area (Fig 2.7, Table 2.2). The Stage 2 and 3 levels match fairly closely the same pattern as for the Namoi/Gwydir but at a lower level. The trend to steadily increasing resistance in all three Stages over time, noted both in the Namoi/Gwydir and at Emerald, was also evident in the Inverell Refugia (Fig 2.7).

### *Dilution potential of Refugia*

The index of Total Season Selection Pressure (the increase in pyrethroid resistance between the start (Stage 1) and the end (Stage 3) of the season, (Table 2.5) is influenced by a complex interaction of factors selecting for and against resistance. Table 2.5 and Fig 2.11 compare the relative impact of an operational factor favouring selection (pyrethroid use) and two ecological factors (summer rainfall and sorghum production) favouring dilution by susceptibles from the Refugia. Pyrethroid use and summer rainfall correlated poorly with the Total Season Selection Pressure, while the best correlation was clearly with sorghum production (Fig 2.11). When sorghum production was above average, either because of good summer rain (1983/84 & 1987/88) or a large area sown (1986/87), dilution by susceptibles resulted in the lowest selection indices, while in dry years with sorghum production below average (1984/85, 1985/886 & 1988/89), Selection indices reflected closely the pyrethroid use in the sprayed cotton areas, without the confounding influence of immigration (Table 2.5 & Fig 2.11).

### *Endosulfan resistance - Namoi/Gwydir*

Each season showed a similar pattern with the largest increases (up to 2.4 fold) between Stages 1 and 2, with only smaller increases or none, between Stages 2 and 3 (Table 2.2). These moderate mid/late season resistance levels always returned to low levels by the beginning of the following season (Table 2.2 & Figs 2.6 & 7). Unlike the pyrethroids, there was no trend to increasing resistance levels in any Stage over time.

### *Endosulfan resistance - Emerald*

The resistance pattern at Emerald was similar to the Namoi/Gwydir but with some differences. The increases between Stages 1 and 2 were generally higher at Emerald (up to 6.8 fold, Table 2.2) despite similar Stage 1 selection pressure (Appendix 3). The average Stage 3 figures at Emerald indicated little or no change between Stages 2 and 3 (Table 2.2), whereas the weekly data indicated a clear response to Stage 2 selection pressure with sharp Stage 3 peaks (35-45%) in 3 out of 4 seasons (Fig 2.6). Similar high levels, were only reached in 1 out of 4 seasons in the Namoi/Gwydir (Fig 2.6). The longer Stage 3 season at Emerald would have masked these transient high levels when averaged over the entire Stage 3 period. As in the Namoi/Gwydir, these moderate to high mid/late

season resistance levels always returned to low levels by the beginning of the following season and there was no trend to increasing resistance levels in any Stage over time (Table 2.2, Figs 2.6 & 7).

### *Endosulfan resistance - Inverell Refugia*

Resistance remained low and relatively constant throughout the whole study and did not reflect the increases recorded in the nearby Namoi/Gwydir cotton area (Table 2.2 & Fig 2.7).

### *Resistance by Host Crop*

During collecting trips, eggs were sampled from whatever hosts were available at the time. Obviously, few alternate hosts were available in the Namoi/Gwydir cotton monoculture but various crop hosts and weeds were available at Emerald and in the Inverell Refugia. Table 2.7 indicates the periods when sufficient samples of more than one host were able to be collected concurrently. Quite clearly, resistant moths did not discriminate between hosts as there was no occasion where either pyrethroid or endosulfan resistance levels differed between the various crop and weed hosts (Table 2.7).

### *Comparison of Sampling Errors*

The between site binomial standard error was on average slightly higher than the pooled binomial standard error for fenvalerate, but not for endosulfan or the fenvalerate/piperonyl butoxide mix (Table 2.1).

## Discussion

### *Monitoring technique*

The monitoring technique employed in this study (discriminating dose screening of larvae reared from field collected eggs) proved extremely successful and had a number of advantages over other techniques. It was found to be extremely sensitive in detecting even small changes in resistance which could then be correlated with various operational and ecological factors. This would not have been possible if the classic resistance monitoring technique (fully bioassay of lab reared F1 progeny) had been employed (see Chapter 3). This no doubt, was due in part to the improved statistical efficiency of the discriminating dose technique (Roush & Miller 1986) but also to the fact that the technique allowed assay of individuals unchanged genetically from the field. The importance of bioassaying material direct from the field to avoid altering resistance frequencies during laboratory culturing, has been noted by a number of authors (Dennehy 1987, Roush & Miller 1986, Boggild & Keiding 1958). Field material can also be lost during lab culturing through prior parasitism (eg. Suckling et al. 1987), disease, escapes, rearing deformities and low copulation rates (eg. Topper 1987) which can often mean that putative and actual population numbers are generally quite divergent. This technique avoided the loss of field collected material to parasitism and disease, except for a small amount of parasitism by the egg parasites *Trichogramma* sp. and *Trichogrammatoidea* sp. (especially at

Emerald) and the egg/larval parasite *Chelonus* sp. (early season only). The technique allowed screening under closely controlled standard conditions (temperature, weight, diet) and also avoided the possibility of prior exposure to sub lethal doses, all of which can be major variables with the assay of field collected moths. The technique also catered quite easily for the assay of field material from remote sites at a centralised testing laboratory, quite impossible with moth testing (Forrester 1990). It also allowed culling of the coincident sympatric *Heliothis punctigera* species, which is not possible with any of the techniques involving the screening of neonates reared from field collected eggs. These latter self dosing foliar residue tests are also subject to avoidance behaviour problems (Brown & Brogdon 1987) and have, at least for *Heliothis armigera*, been shown to be less efficient than the precision dosing topical larval test (McCaffery et al. 1988).

Because of the high migratory ability of *Heliothis armigera* (Farrow & Daly 1987, Daly & Gregg 1985), no meaningful trends could be found by correlating resistance and insecticide use on an individual property basis. Consequently, each study area was treated as one large 'Heliothis farm' and collection data for properties were pooled over set periods of time (either weekly or by Stage). Such pooling was also found necessary when analysing data for the considerably less mobile housefly (Gibson 1981). The need to pool samples from a large area because of migration between properties with different selection regimes, precluded the possibility of incorporating an effective 'control' area of unregulated insecticide use into the study (discussed further in Forrester 1990).

No attempt was made to convert the % resistance data (i.e. % of larvae surviving the discriminating dose) to gene frequencies, as this was considered inappropriate for a number of reasons. There were multiple genes involved with at least three resistance mechanisms (Gunning 1988, Sawicki & Denholm 1989) and the interaction of these genes was and is still, unknown. There was also a variable overlap of the susceptible and heterozygote lines for the principal metabolic resistance mechanism (Daly 1988, Daly & Murray 1988), with the degree of this overlap possibly depending on the genetic background (eg. Busch-Petersen & Wood 1986). Given all these difficulties, it was decided not to attempt to convert the % resistance data to gene frequencies in this study, as there was insufficient information available on the genetics of any of the resistance mechanisms or their various combinations.

The comparison of the between site and pooled binomial standard errors yielded some interesting information for designing possible future monitoring programmes. Because of funding restrictions, more economical monitoring methods are being continually sought. One such possibility is to remove the need to handle samples separately in the laboratory by combining collections for a set time period (say weekly intervals). This would then lower the labour requirement for sample and data processing without any loss in precision, at least for endosulfan. The slightly higher (8%) error level for the fenvalerate between site binomial standard error over the pooled, indicates that ideally, collecting sites should be kept separate for this chemical. The reason for the larger between site variation for fenvalerate over endosulfan, can probably be attributed to the noted repellent properties of the pyrethroids (Sawicki et al. 1989, see also Chapter 7 and references therein). The slight loss in precision

incurred by adopting the simpler pooled error estimate would only be significant for the pyrethroid screen component of the programme and this disadvantage could well be offset by a reduction in programme running costs.

The confirmation that resistant moths do not discriminate between host plants also indicates another possibility to reduce costs. One of the biggest problems in the current programme is the collection and partial processing of large numbers of the unwanted coincident sibling species *Heliothis punctigera*, especially in Stage 1. All of the dicot hosts (eg. cotton, sunflowers, soybean) attract both species but the graminaceous hosts (sorghum and maize) attract only *Heliothis armigera*. Maize is also attractive for a long period with eggs being laid even at the young vegetative stage right up until silking. There exists an excellent opportunity to increase the sampling efficiency of this programme by the sowing of sentinel maize crops, especially in the *H. punctigera* dominant Stage 1 period and especially in the Namoi/Gwydir cotton monoculture study area. This could then also introduce the possibility of further cost savings in lab rearing by allowing the screening of neonates reared from field collected eggs. This is not possible now because of the uncertaintity of the species composition of eggs collected from the predominant dicot host crops.

The success of the monitoring programme in documenting the impact of the newly introduced Strategy, has been instrumental in maintaining the Strategy's excellent compliance rate. Because of the sensitivity of the monitoring technique, Strategy users have, in addition to verifying the anticipated impact of the Strategy, been able to identify problems, adjust their management practices accordingly and assess the effectiveness of these initiatives (Forrester 1990). Without this ability, growers, resellers, consultants and the agrochemical industry would not probably have had the confidence to continue with the Strategy and the compliance rate would probably have gradually declined over time. Indeed, it is suggested that the success of this monitoring technique supports Dennehy's (1987) comment that an effective and efficient monitoring methodology is essential to make Resistance Management Strategies "implementable and verifiable".

## *Pyrethroid resistance*

There were clearly two separate pyrethroid selection factors operating: one manifesting itself within the Stage 2 window, immediately following the first pyrethroid use; the other showing up in early Stage 3, about a generation after the first pyrethroid use. The increase in early Stage 3 can be explained by selection of larvae, which would have developed through to egg laying moths by early Stage 3. However, the immediate increase within the Stage 2 window was unexpected and appeared too quickly to be explained by larval selection. Since there is no evidence of cross resistance between endosulfan and pyrethroids (see Chapter 4), endosulfan selection on larvae in Stage 1 could not have been the cause. The second possibility examined was selection of moths prior to egg laying. This was not considered at first as it was a generally held belief (see references in Chapter 7) that nectar feeding adult lepidopterans would not express metabolic resistance. However, it was quickly shown that *Heliothis armigera* moths did indeed express pyrethroid resistance and that this was the cause for the

rapid increase in resistance within weeks of the first pyrethroid use (see Chapter 7). In fact, this adult selection was very sensitive to selection pressure as was seen in the 1986/87 Namoi/Gwydir Stage 2 window when pyrethroid resistance dropped dramatically in response to a switch from pyrethroids to other chemical groups.

The higher Indices of Adult Selection at Emerald, despite often lower selection pressure, are very interesting as they indicate the impact of cropping pattern on adult selection. Emerald is a mixed cropping area with maize the main alternate crop to cotton. *Heliothis armigera* lays on silking maize in late November/early December at Emerald, producing moths 5-6 weeks later, right at the start of the Stage 2 pyrethroid window. These moths emerge from the senescent maize blocks and fly direct to the neighbouring cotton where they are immediately selected with pyrethroids. The selection in this ecological system would occur mostly before mating whereas in the Namoi/Gwydir cotton monoculture, most moths would immigrate into the cotton already mated. It has been recognised for some time that resistance evolves more rapidly when selection precedes mating (Wood & Bishop 1981, Mani & Wood 1984, Rosenheim & Hoy 1988) so this would explain the higher rates of adult selection at Emerald. This explanation also fits nicely with the observation that the Indices of Adult Selection at Emerald correlate very well with the area of maize grown, being highest when maize accounted for approximately a quarter of the cropping area and lowest when the maize area dropped to 10% or less.

Whilever the Stage 2 window was 42 days, the Stage 2 adult selection peak ran on into the start of the larval selection peak, giving the impression of one sharp Stage 3 peak. There were two exceptions to this. One was in the 1986/87 Namoi/Gwydir season where, as discussed earlier, the adult selection peak was cut off early and the Stage 3 larval peak, although occurring at the normal time in early March, could be easily distinguished from the earlier adult selection peak because of the trough in late Stage 2. The other exception was the 1989/90 season when the Stage 2 pyrethroid window was shortened to 35 days to avoid the double selection that was occurring in the 6th week (see Chapter 1). This achieved the desired impact as the two selection peaks were clearly separated, with the trough occurring in early Stage 3. Thus the initiative to shorten the pyrethroid window by one week was shown to be a successful delaying tactic.

There are many factors (genetic, biological and operational) which can affect resistance development (Georghiou 1983, Georghiou & Taylor 1986). Often these factors can interact, producing seemingly conflicting results. For example, seasons with the highest pyrethroid use do not necessarily result in the highest resistance levels. The best indicator of the season's selection pressure is the increase in pyrethroid resistance between the start (Stage 1) and the end of the season (Stage 3). This is better than using the Stage 3 peak alone, as it accounts for seasonal variation in starting levels. Using this index, it was found that immigration of susceptibles from the unsprayed refugia was the most important factor affecting resistance levels. The operational factor (pyrethroid use) was also important, but clearly more so in seasons when immigration from the refugia was negligible.

A decline in insecticide resistance over the non selecting regression interval, is a basic tenet of resistance management by rotation (May & Dobson 1986). Such a situation was confirmed in this study

where Stage 1 spring/early summer levels were always significantly lower than the previous Stage 3 autumn levels in both the Namoi/Gwydir and Emerald. Interestingly, the degree of these declines correlated well with the level of cotton stubble cultivation which in turn could be neatly correlated with Futures forecasts in the price sensitive cotton industry. Cotton stubble has been shown to be the major source of overwintering *Heliothis armigera* pupae in the Namoi/Gwydir and because of the intensity of spraying in this area, also has the lowest pupal parasitism rates (Fitt & Daly 1990). Murray and Cull (1984) also found high densities of overwintering pupae of *H. armigera* at Emerald (up to 30/square metre) in the winter following the 1983 pyrethroid field failures. In addition to the high numbers and low parasitism rates, these pupae would also have derived from eggs laid during the peak resistance period in Stage 3. Thus the overwintering population under cotton constitutes the major source for the carryover of resistant *H. armigera* from one season to the next. Left undisturbed, these pupae provide the nucleus for the following season's pyrethroid resistance problems. The potential for overwintering sites to provide foci for resistance to develop and spread has also been recognised by Roush & McKenzie (1987) and Denholm et al. (1985). Cultivation of winter cotton stubble has been shown to be an effective means of killing these overwintering pupae (Fitt & Forrester 1987, Fitt & Daly 1988) and this study has resulted in the addition of a supplementary Strategy guideline to sample overwintering pupae under cotton stubble and to cultivate if necessary (see Chapter 1). Indeed, Hearn (1975) suggested that the greater survival of *H. armigera* under undisturbed ratoon cotton crops in the Ord, may have contributed to the resistance problem there as well. The increasingly popular practice of direct drilling or aerial sowing of winter crops into undisturbed cotton stubble (Table 2.6) should only be undertaken in situations where overwintering pupal populations are low or absent. Cultivation of overwintering resistant pupae should always remain a significant component of the Australian Resistance Management Strategy. The overwintering pupal stage is the weak link in the *Heliothis armigera* life cycle as it can remain vulnerable to simple 'resistance proof' physical control measures for almost 6 months. Growers will need to consider cultivation of overwintering pupae much more seriously in the future as the survey results indicate that as much as one third of the cotton residues are left ineffectively cultivated and that growers' current stubble management and crop rotation decisions change little from season to season, except in response to large changes in cotton price forecasts. The value of cultural controls in slowing down resistance has been recognised previously (Macdonald et al. 1983, Harris et al. 1982, Hammock & Soderlund 1986) and should be exploited more assiduously in the Australian Strategy.

As mentioned previously, Stage 1 spring/early summer resistance levels were always lower than those in the preceding Stage 3 autumn. The most likely reason for this decline in resistance is dilution of local resistant populations, derived from overwintering pupae, by immigration of individuals from more susceptible populations in spring (Daly et al. 1988). Commenting on a similar situation with *Heliothis virescens* in the USA, Plapp et al. (1990) suggested that reproductive disadvantage of resistant moths was the cause but ignored completely the equally plausible explanation of simple dilution. Stage 1 resistance levels in the early years of the Strategy showed little variation even during

the multiple generation Stage 1 periods at Emerald. However, as resistance levels have increased over time, so has the variation in Stage 1 levels in both areas with quite often large differences in resistance levels even between adjoining weeks. These large fluctuations in resistance within the current Stage 1 periods can probably be explained by a complex mixing of resistant individuals emerging from a variable diapause with susceptibles immigrating at particular times. While resistance levels were low, the local overwintering resistant populations would have had only a minimal impact on spring/early summer resistance levels. However, as resistance levels have increased, the influence of these local resistant populations would have also increased resulting in large fluctuations in resistance levels according to the magnitude and timing of these emergence and immigration events.

The longer season in the northern Emerald study area allows 2-3 extra generations per year and consequently longer regression intervals (May & Dobson 1986). Theoretically, this should have resulted in more effective resistance management at Emerald but there was little difference in resistance levels between the short season Namoi/Gwydir and long season Emerald areas. However, it is possible that any gains from this factor could have been offset by the higher *Heliothis armigera* pressure and/or premating selection which occurs at Emerald.

The 1985/86 Stage 1 resistance levels at Emerald (6.8% average overall) are particularly interesting as they indicate the level of pyrethroid resistance after two seasons of non use. This would seem to be the base level which can be easily achieved in a reasonable time frame. Interestingly, Weinzierl et al. (1990) found a similar level of residual pyrethroid resistance (4-8%) in hornfly in Illinois after 2 years without pyrethroids. Withdrawal of pyrethroids for longer periods would probably result in little, if any, improvement. Pyrethroid resistance dropped quickly from high levels but showed much slower declines at lower resistance levels, also noted for DDT resistance in anopheline mosquitoes in India (Curtis et al. 1978).

It is well accepted that a refugia of susceptibles slows the evolution of resistance (Georghiou & Taylor 1976 & 1986, National Academy Press 1986, Leeper et al. 1986, Roush & Croft 1986, Mason et al. 1989). However, it has also been suggested that as the refugia becomes contaminated, the treated area will move sharply from susceptibility to resistance (May & Dobson 1986). This would seem to be happening in this study as the resistance levels in the unsprayed refugia approach those found in nearby cotton areas. This declining effectiveness of the refugia as a source of susceptibles for dilution has resulted in a clear and steady increase in resistance levels in both cotton areas over time. As mentioned previously, there have certainly been decreases in resistance during the non-use regression intervals but the increases due to larval and adult selection have outweighed these decreases, resulting in a fluctuating but nonetheless inexorable progression to increased resistance. This escalatory effect was also noted by Georghiou et al. (1973) for organophosphate and carbamate resistance in *Anopheles albimanus* in El Salvador. Thus it is clear that the Australian Strategy has been successful in extending the useful life of the pyrethroids. However, it is also clear that the Strategy alone has not overcome the problem. It has simply bought time (Hammock & Soderlund 1986, Sawicki & Denholm 1987) to allow the discovery, development and implementation of alternative control

measures, both chemical and non-chemical (eg. see work on synergists and resistance breaking pyrethroids in Chapters 9 & 10).

### *Endosulfan resistance*

Endosulfan is one of the key insecticides for control of *Heliothis* spp. in Australian cotton. Endosulfan resistance in *Heliothis armigera* has been a problem since the early 70's (see Fig 1.1 in Chapter 1) and one of the Strategy's aims from the outset was to prevent reselection of this historical endosulfan resistance. Most endosulfan use is targeted early season in Stage 1, although a smaller amount is also used in Stage 2 (see Appendix 3). Therefore, larval selection should be manifested principally in the following generation in Stage 2 with a smaller response in the Stage 3 period. Indeed, this occurred in both the Namoi/Gwydir and Emerald study areas and indicates a similar response pattern to larval selection as with the pyrethroids. However, there was no clear indication of an immediate increase in endosulfan resistance within the Stage 1 period, suggesting little impact of adult selection with endosulfan. The endosulfan responses at Emerald were generally higher than in the Namoi/Gwydir, despite similar selection pressure. This can probably be attributed to the higher *Heliothis armigera* pressure at Emerald, particularly during the period of intense endosulfan use in Stage 1.

Endosulfan resistance in the unsprayed refugia at Inverell remained low and relatively constant throughout the season and, as opposed to the situation with pyrethroids, did not reflect closely the increases recorded in the nearby Namoi/Gwydir cotton area. This may have been because endosulfan is not considered to possess the irritant and repellent properties of the pyrethroid insecticides which promote effective dispersal of pyrethroid resistant moths (see Chapter 7 and references therein).

There was also no evidence of a trend to increasing endosulfan resistance in any area over time. This contrasts sharply with the gradually deteriorating resistance mentioned previously for the pyrethroids. Possible reasons for this are discussed below.

### *Comparison of pyrethroid & endosulfan resistance*

The deteriorating pyrethroid resistance situation has been attributed principally to the declining effectiveness of the refugia as a source of susceptibles for dilution of pyrethroid resistance. However, it would seem that the refugia still remains useful as a source for dilution of endosulfan resistance and that this is the reason for the greater success of the Strategy in managing endosulfan resistance. A number of factors probably contributed to the maintenance of relative endosulfan susceptibility in the refugia. Firstly, despite endosulfan being the most frequently used insecticide in cotton (see Appendix 3), it is used mainly in Stage 1 when *Heliothis armigera* numbers are at their lowest. Therefore, actual selection pressure against the *armigera* species could well be lower for endosulfan than that for fewer pyrethroids targeted on higher *H. armigera* populations in Stage 2. This would have resulted in effectively lower endosulfan selection pressure despite its greater use. This is probably the simplest explanation and is

consistent with the finding that endosulfan resistance responses were higher at Emerald where *H. armigera* selection pressure would have been more intense.

However, there are other possible explanations for the difference between the pyrethroids and endosulfan. Endosulfan resistance could incur a fitness deficit, whereas pyrethroid resistance does not. Although the latter has been researched in this study (see Chapter 5), no work has been done on the fitness of endosulfan resistant individuals, so this explanation cannot be discounted. In addition, adult selection could also be less important for endosulfan than pyrethroids and in fact, there is some indication of this in this study. Thus, as suggested by Georghiou & Taylor (1976) and Tabashnik & Croft (1982), it would be expected that pyrethroid selection on two life stages (moths & larvae) would result in worse resistance problems than endosulfan selection on only one life stage (larvae).

A further possible explanation is a difference in the dominance of the major resistance genes. It is widely recognised that resistance is much more easily controlled if it is recessive (Curtis 1985, Georghiou 1983, Croft & van de Baan 1988, Wood & Bishop 1981, Curtis et al. 1978). The major pyrethroid resistance mechanism in Australian *Heliothis armigera* (metabolic detoxification) has been found to be controlled by a semi dominant gene (Daly 1988, Gunning & Easton 1987). However, the genetics of the endosulfan resistance gene remain unknown, although other cyclodiene resistance genes in various organisms have been shown to be intermediate, ranging from incompletely recessive to incompletely dominant (Brown & Pal 1971, Plapp 1986, Wood & Bishop 1981, Bonner & Yarbrough 1987, Busch-Petersen & Wood 1986, Oppenoorth 1985). If a similar situation occurs with endosulfan resistance in *Heliothis armigera*, then it is quite possible that the greater dominance of the major pyrethroid resistance gene could also explain the more serious pyrethroid resistance problems.

The Strategy has been much more successful in managing endosulfan than pyrethroid resistance. It would be very useful to attempt to identify the reasons for this to assist in the design of new or modification of current Strategies. A number of possible explanations have been put forward here, including effectively lower selection pressure, fitness deficit, fewer life stages selected and less genetic dominance. However, it has not been possible from this study to distinguish the relative importance of these factors or their possible interactions.

It was suggested previously that effectively lower selection pressure could well be a major factor favouring the lower endosulfan resistance levels. If indeed this is so, then any change in the endosulfan use pattern should be treated with caution. This would be particularly so if there was any increased use of endosulfan on the higher *Heliothis armigera* populations which occur later on in the Stage2 period. Up until now, endosulfan has had only a small but consistent use in Stage2 (15-20% of Stage2 sprays, see Appendix 3) being used mainly as an alternative to pyrethroids to avoid mite flare. However, as the pyrethroid resistance situation has deteriorated, growers and consultants have turned to endosulfan in Stage2 as a break chemical or cleanup spray for pyrethroid failures especially at Emerald where endosulfan now accounts for almost 40% of Stage2 sprays (see Appendix 3 ). This increased use of endosulfan in Stage2 has effectively resulted in an extension of the endosulfan selection period to two consecutive generations (three in the longer season Emerald area). If

endosulfan continues to be increasingly used in Stage2, the greater selection pressure could well tip the balance and endosulfan resistance could follow the same trend as pyrethroid resistance. Thus it is critical to minimise the use of endosulfan in Stage2 and the best way to do this is to preserve the efficacy of the pyrethroids (various options discussed in Chapters 8, 9 & 10). If management of pyrethroid and endosulfan resistance is to be successful, it will be essential to strike a fine balance between using endosulfan to manage pyrethroid resistance and vice versa.

### *Mixtures versus rotations*

The Australian Strategy is based on the rotation of unrelated chemical groups on a per generation basis alongwith a strong recommendation for the use of ovicidal mixtures (see Chapter 1). The rotation component of this Strategy has proven to be the most important as the use of mixtures has declined for a combination of reasons (see Appendix 3). There have been many theoretical studies demonstrating that such rotation strategies can delay resistance (eg. Georghiou & Taylor 1977, Via 1986, Comins 1986) as well as an increasing number of practical studies indicating the same (eg. Macdonald et al. 1983, Flexner et al. 1988, Immaraju et al. 1990). This study shows that the Australian Strategy, based on alternation by generations in a co-ordinated way across a region to avoid mosaic effects (Roush 1989), has also been successful in delaying resistance.

The resistance literature is replete with discussions on the merits of mixtures versus rotations (Georghiou 1983, Comins 1986, Nat. Academy Press 1986, Leeper et al. 1986, Mani 1985, Curtis 1985 & 1987, Mallett 1989, Roush 1989, Holloway & McCaffery 1988) but as Tabashnik (1989) so rightly points out, most concentrate on medical and veterinary pests (particularly mosquitoes and houseflies) and ignore the problems that mixtures can create in cropping ecosystems (eg. disruption of biological control, induction of secondary pests, selection for resistance in secondary pests, increased costs etc). The declining reliance on mixtures in the Australian Strategy, support Tabashnik's (1989) comments on the limited role for mixtures for management of insecticide resistance in crop pests.

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## Resistance Monitoring Study Sites : Eastern Australia

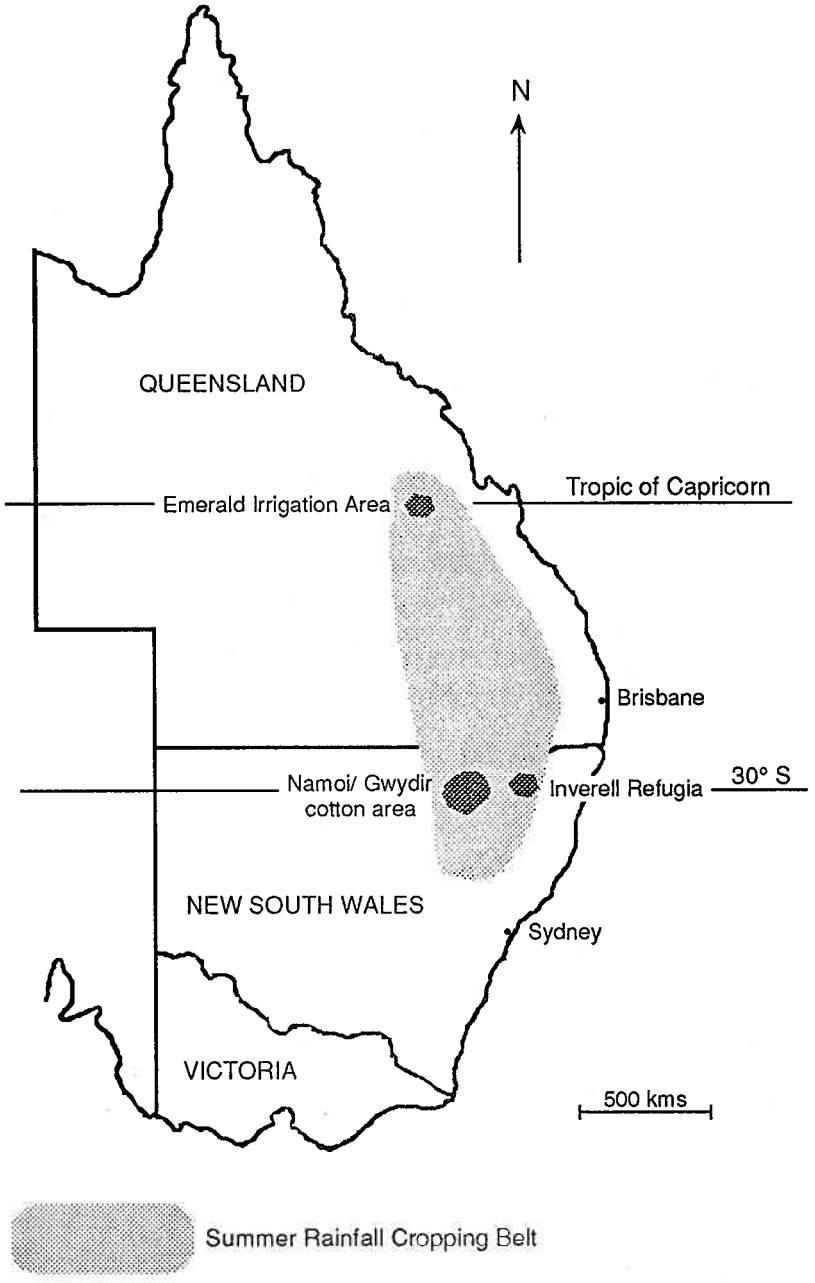


**Fig. 2.1** Location of the three resistance monitoring study sites within the eastern Australia summer rainfall cropping zone; the Emerald Irrigation Area of central Queensland (an irrigated mixed cropping system, mainly cotton), the Namoi & Gwydir river valleys of northern New South Wales (an irrigated cotton monoculture) and an unsprayed refugia area of dryland (raingrown) alternative host crops (mainly maize, sorghum & sunflowers), centred just west of Inverell in northern New South Wales.

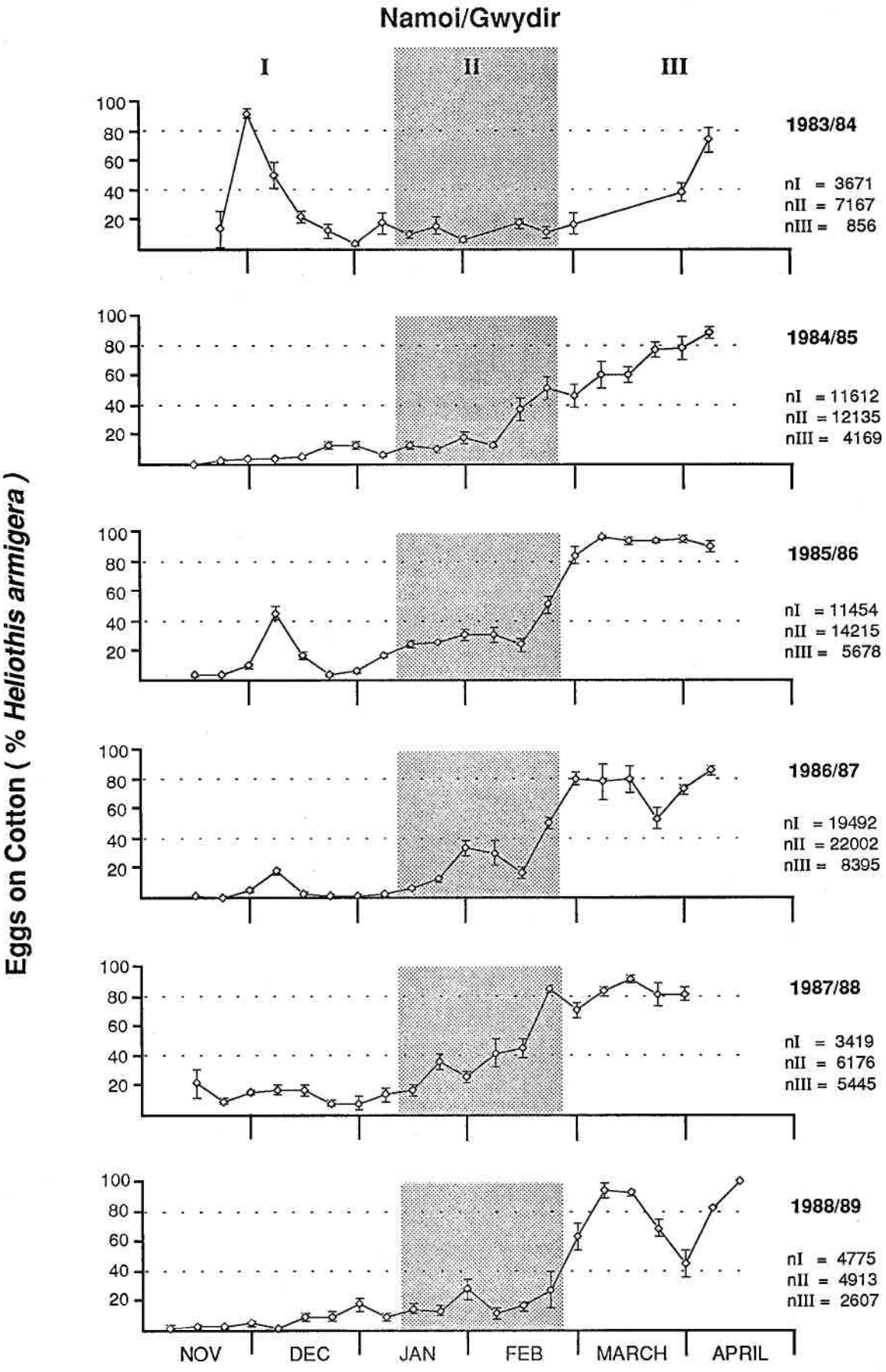


**Fig. 2.2** Average weekly percent *Heliothis armigera* (± standard error of mean) reared from eggs collected off cotton in the Namoi and Gwydir river valleys of northern NSW. nI, nII,nIII = the total number of larvae (*H. armigera* plus*H. punctigera* ) reared for each Stage (**I, II & III** ) of the six seasons since the introduction of the Resistance Management Strategy.

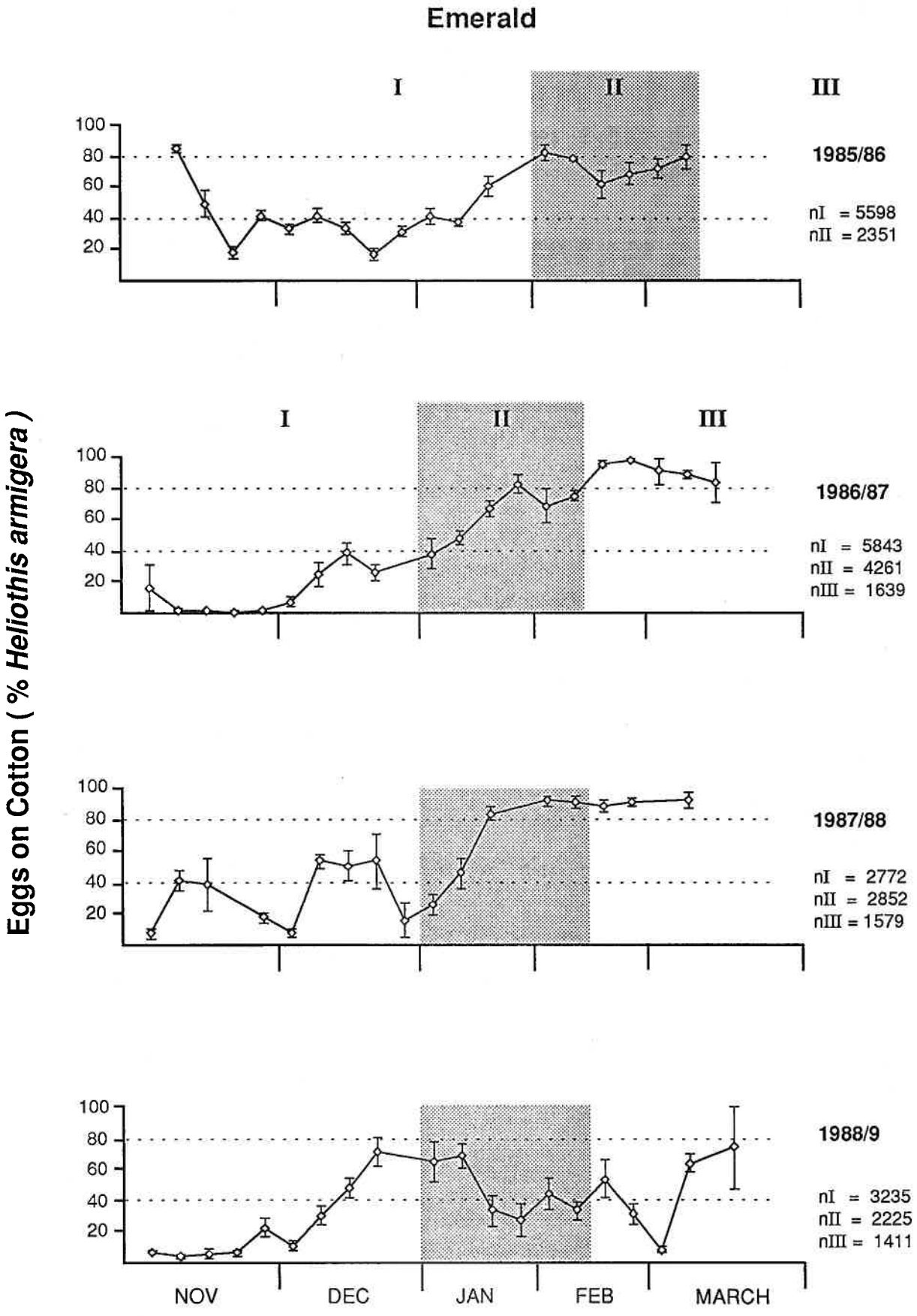


**Fig. 2.3** Average weekly percent *Heliothis armigera* (± standard error of mean) reared from eggs collected off cotton in the Emerald Irrigation Area of Central Queensland. nI, nII,nIII = the total number of larvae (*H. armigera* plus*H. punctigera* ) reared for each Stage (**I, II & III** ) of the past four seasons of the Resistance Management Strategy.

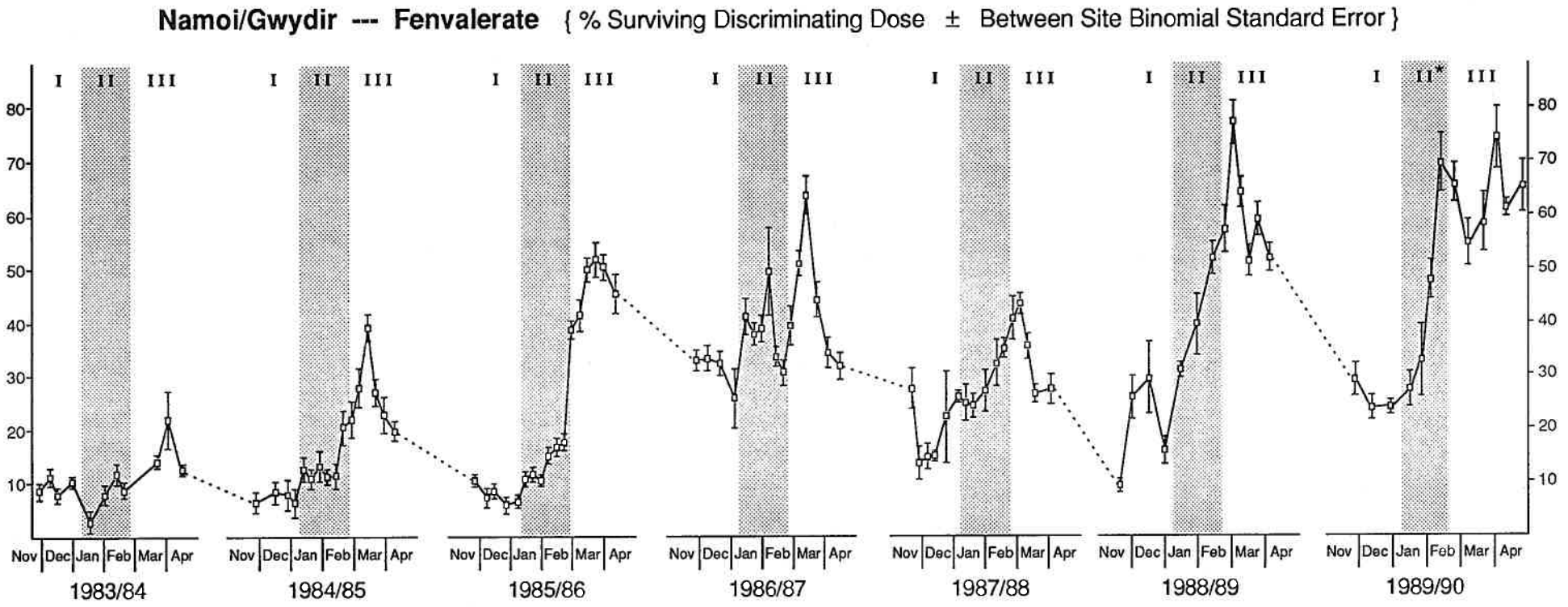


**Fig. 2.4** Weekly pyrethroid resistance in *Heliothis armigera* from the Namoi and Gwydir river valleys of northern New South Wales for the 7 seasons since the introduction of the Resistance Management Strategy (for Stages I, II and III). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the fenvalerate discriminating dose (0.2 micrograms per 30-40 mg larva).

* 1989/90 Stage II window 35 days duration; all others 42 days.

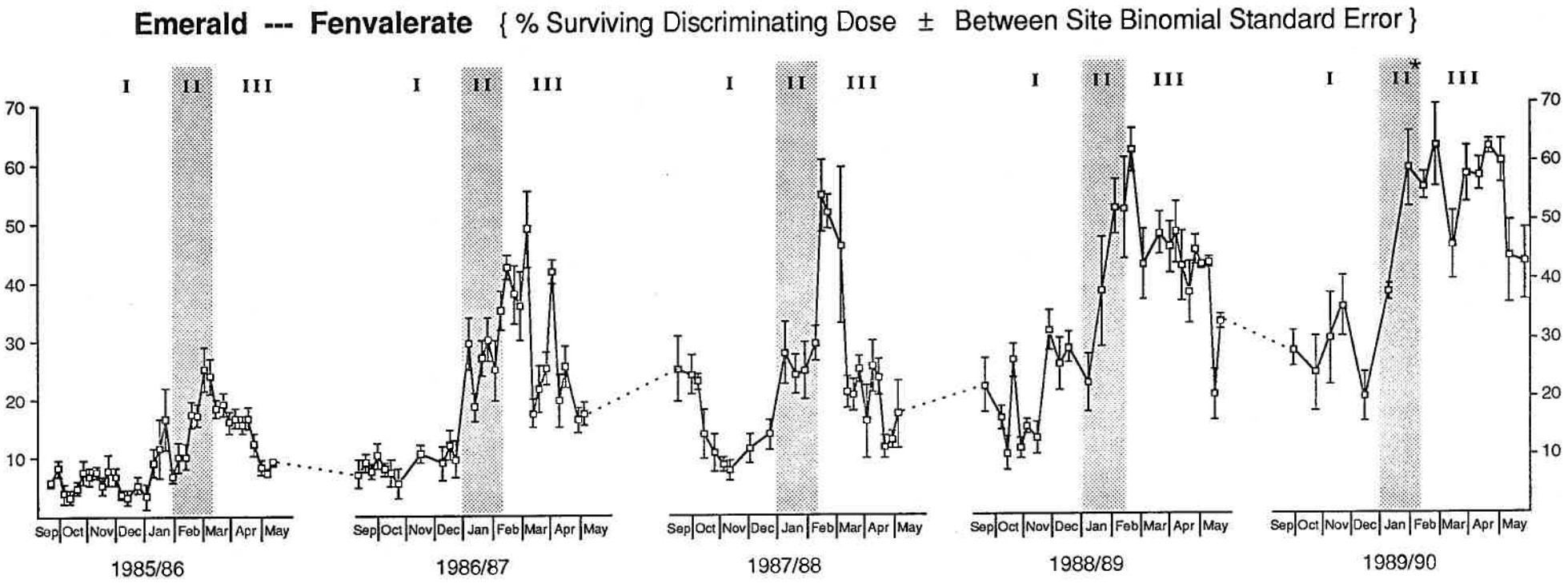


**Fig. 2.5** Weekly pyrethroid resistance in *Heliothis armigera* from the Emerald Irrigation Area of central Queensland for the past 5 seasons of the Resistance Management Strategy ( for Stages I, II and III). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the fenvalerate discriminating dose (0.2 micrograms per 30-40 mg larva).

*1989/90 Stage II window 35 days duration; all others 42 days.

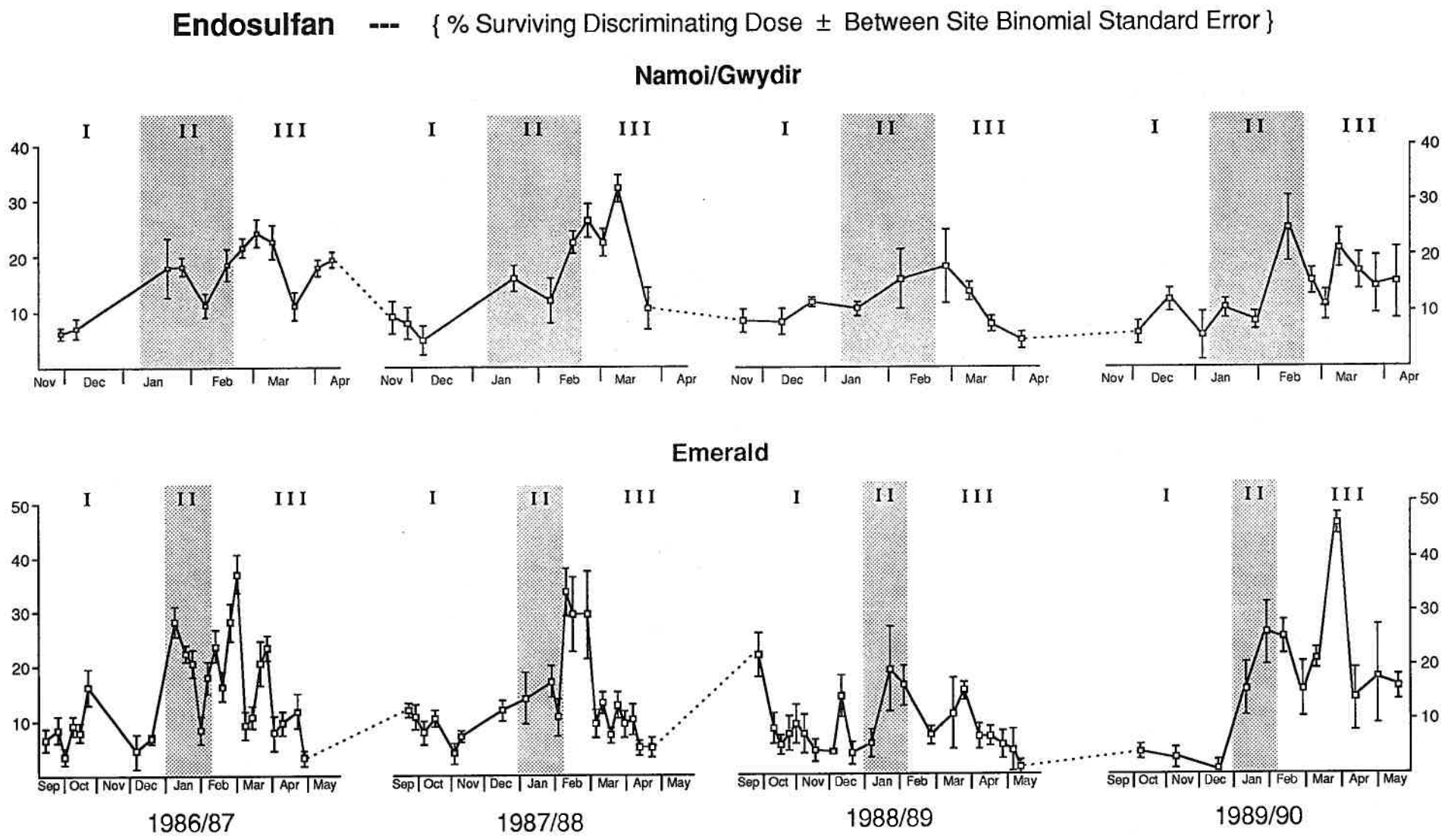


**Fig. 2.6** Weekly endosulfan resistance in *Heliothis armigera* from the Namoi and Gwydir river valleys of northern New South Wales and the Emerald Irrigation Area of central Queensland for the past 4 seasons of the Resistance Management Strategy ( for Stages I, II and III). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the endosulfan discriminating dose (10 µg per 30-40 mg larva).

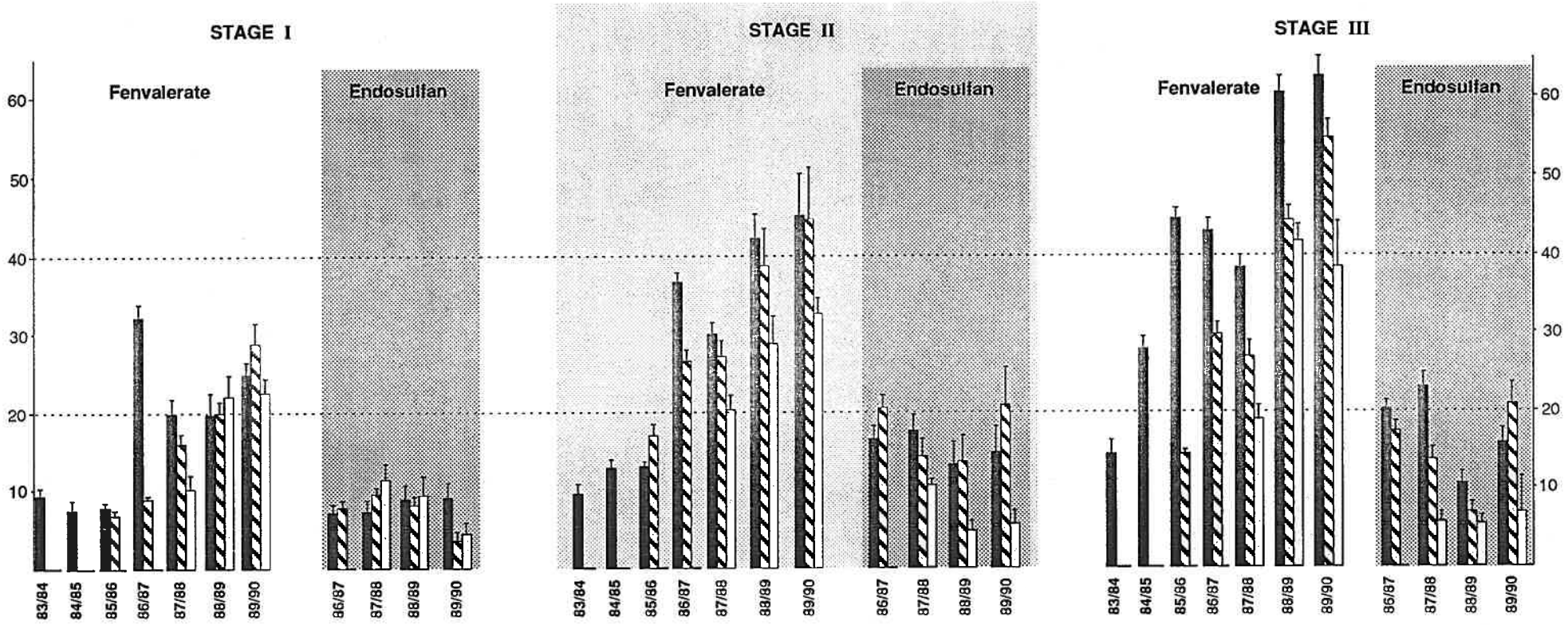


**Fig. 2.7** Average pyrethroid and endosulfan resistance levels in *Heliothis armigera* for Stages I, II & III of the Resistance Management Strategy, for three study areas (the Namoi and Gwydir river valleys of northern New South Wales,the Emerald Irrigation Area of central Queensland and a sample of the unsprayed refugia area centred on Inverell in northern New South Wales). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the discriminating dose (0.2 and 10 micrograms of fenvalerate and endosulfan respectively, per 30-40 mg larva) + the standard error of the mean.

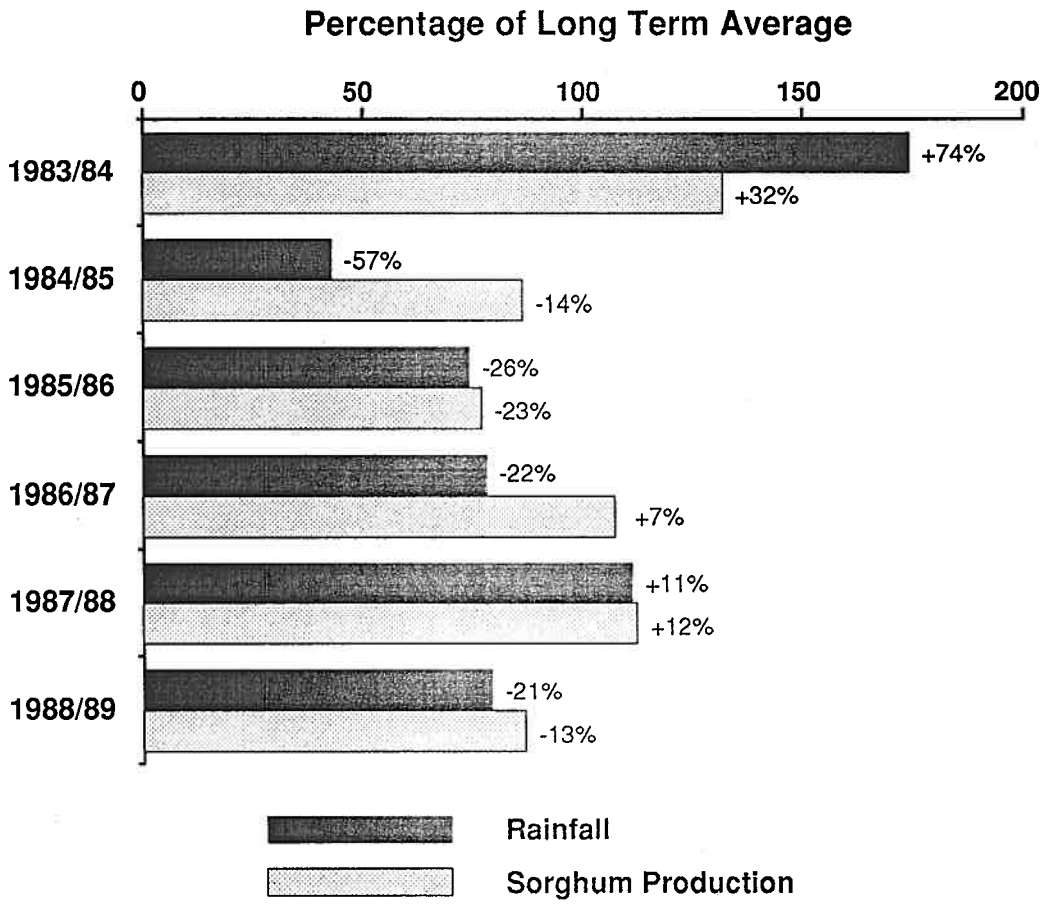


**Fig. 2.8** Stage 3 dilution potential of the unsprayed raingrown cropping area of northwestern NSW, surrounding the Namoi/Gwydir study site, as indicated by the impact of rainfall and its effect on the production of sorghum (the major Stage 2 flowering crop host for *Heliothis armigera* in this area). Rainfall data expressed as the average monthly summer rainfall (December to February) for the six seasons since the introduction of the Resistance Management Strategy, as a percentage of the long term average (64.9mm) for 45 sites spread throughout the area (data source Bureau of Meteorology). Production data expressed as the area's total production as a percentage of the past 6 year average (323,500 tonnes), data source Australian Bureau of Statistics and NSW Agriculture & Fisheries.

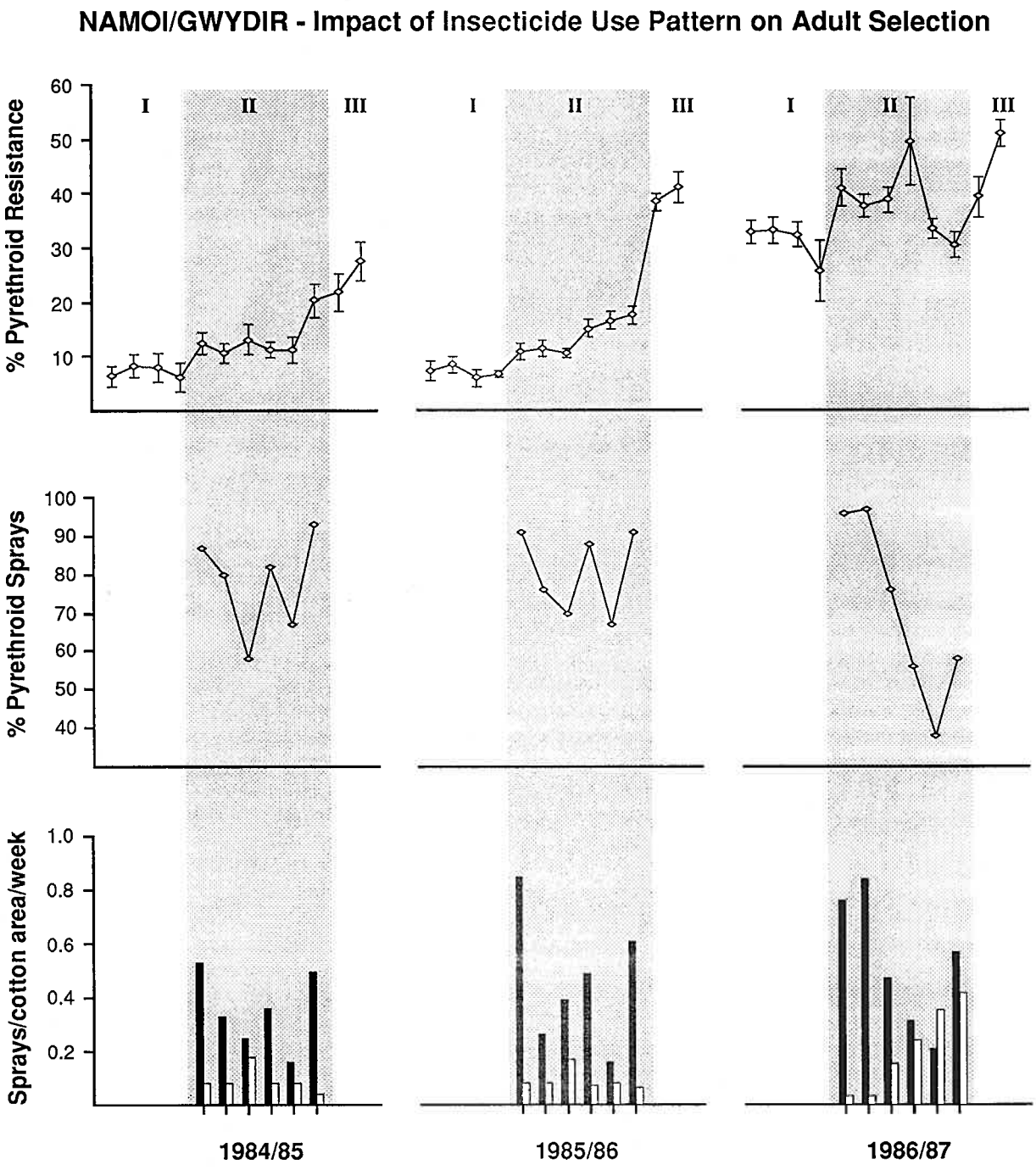


**Fig 2.9** Top Graph.
Weekly pyrethroid resistance ( % of *Heliothis armigera* larvae, reared from field collected eggs, surviving the fenvalerate discriminating dose ± between site binomial standard error) from the Namoi and Gwydir river valleys of northern NSW for the Stage II pyrethroid window, as well as the 4 weeks before and the 2 weeks after.

Middle Graph. Pyrethroid selection pressure expressed as the % of the total area sprayed each week with pyrethroids, for the 6 weeks of the Stage II pyrethroid window.

Bottom Graph. Pyrethroid (■) and non pyrethroid (□) (principally endosulfan, some organophosphates and carbamates) selection pressure expressed as the number of sprays per cotton area per week, for the 6 weeks of the Stage II pyrethroid window.

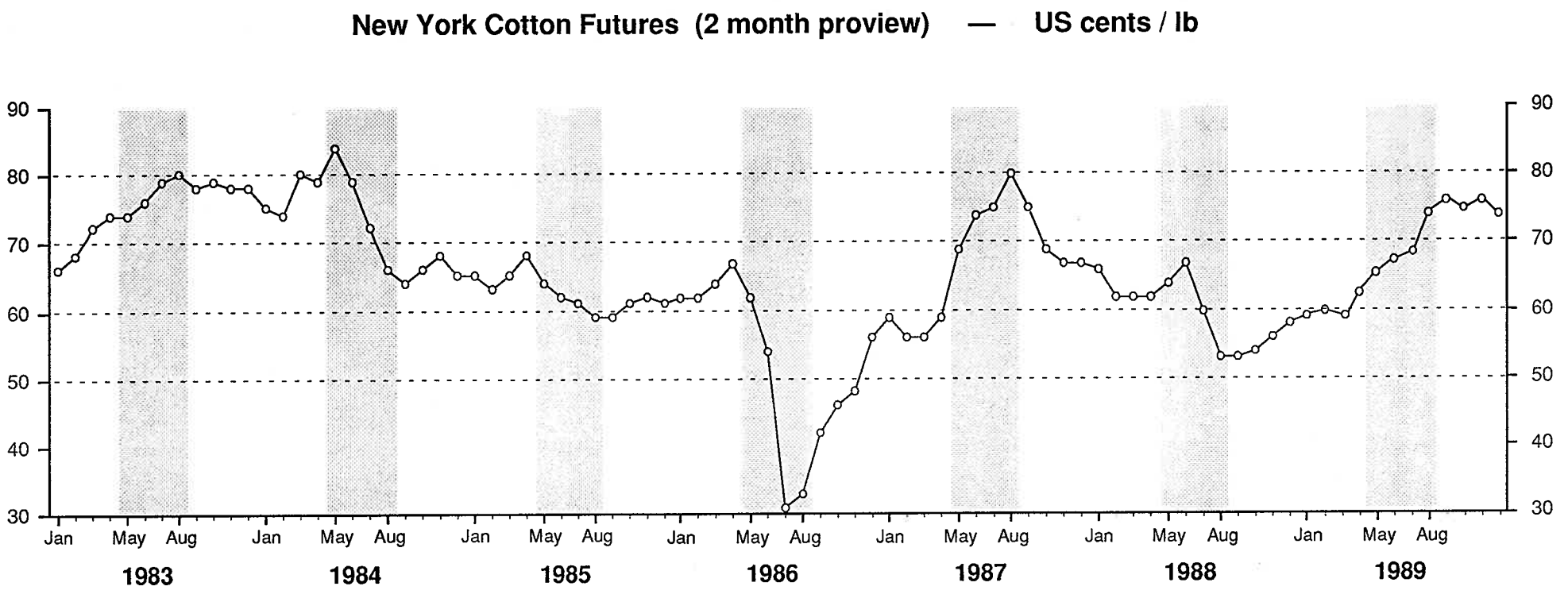


**Fig. 2.10** Average monthly New York Cotton Futures price for the past seven years of the Resistance Management Strategy. Shaded areas represent the 4 month late autumn / winter period (May - August) when Australian cotton growers are making stubble management and crop rotation decisions after the end of picking in March / April. Data source, Namoi Cotton Co-operative, Wee Waa NSW and Merrill Lynch Pty. Ltd., Sydney.

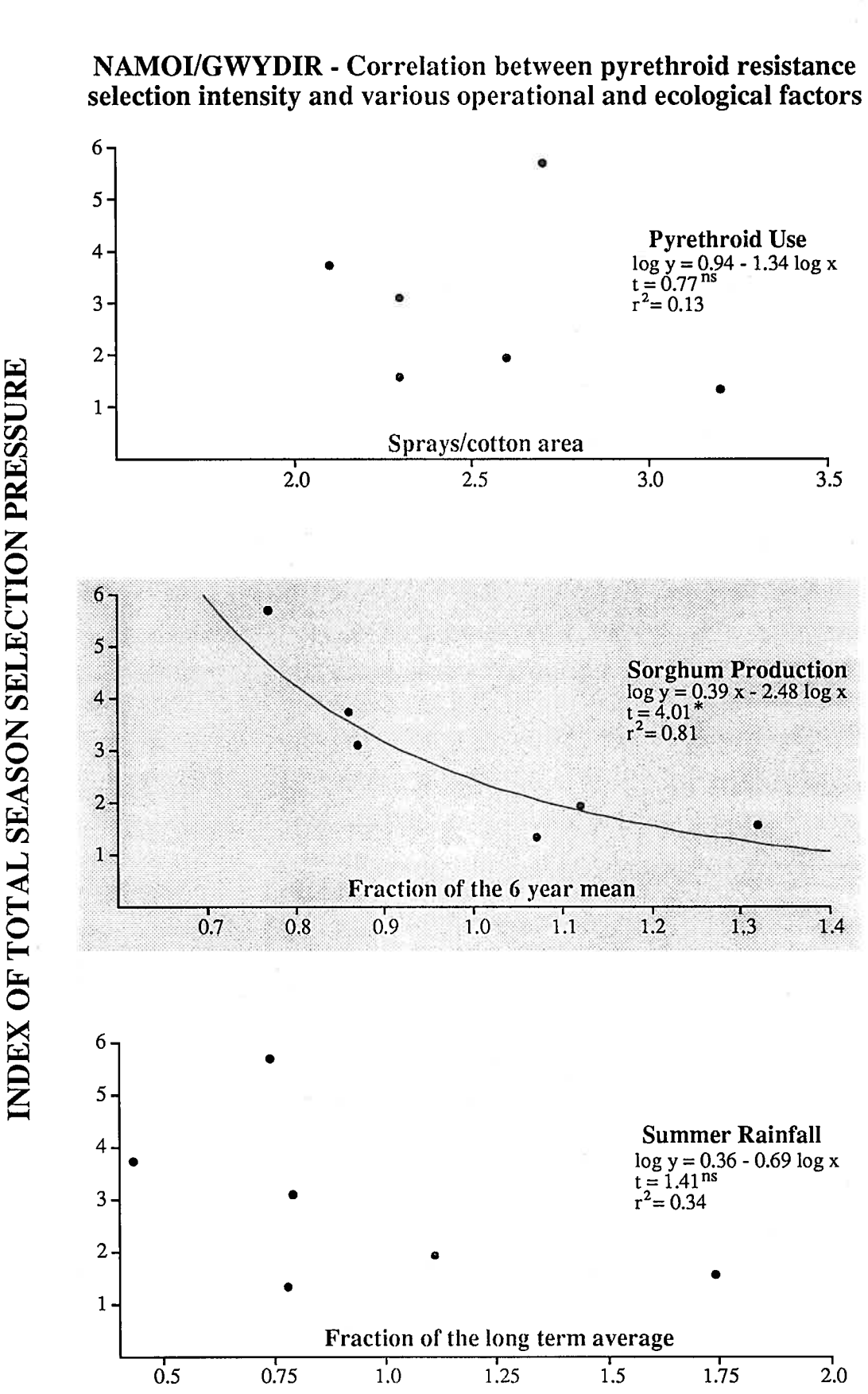


**Fig. 2.11** Index of total season selection pressure for pyrethroid resistance in *Heliothis armigera* from the Namoi and Gwydir river valleys of northern NSW, as influenced by pyrethroid use, summer rainfall and sorghum production. Data (derived from Table 2.5) for the 6 seasons since the introduction of the Resistance Management Strategy. *, ns indicate significant ($p<0.05$) or non significant regression, respectively. ($r^2$ = coefficient of determination).

| Comparison of Sampling Errors | | | |
|---|---|---|---|
| | Between Site ÷ Pooled Binomial Standard Error | | |
| | No. of comparisons | Mean ratio ± s.e. | 99% Confidence interval of mean |
| Fenvalerate | 257 | 1.08 ± 0.030 | 1.00 — 1.16 |
| Endosulfan | 126 | 1.03 ± 0.041 | 0.92 — 1.14 |
| Fenvalerate / Pbo | 116 | 0.93 ± 0.044 | 0.81 — 1.05 |

**Table 2.1** Ratio of the between site binomial standard errors and pooled binomial standard errors for the weekly fenvalerate, endosulfan and fenvalerate / piperonyl butoxide (Pbo) resistance estimates for all three monitoring areas (Namoi/Gwydir, Emerald and Inverell) combined for all years,where:-

$$\text{Weekly Pooled Binomial Standard Error} = \sqrt{\frac{p\,(1-p)}{n-1}}$$

p = proportion of larvae surviving discriminating dose

n = total number of larvae tested that week

$$\text{Weekly Between Site Binomial Standard Error} = \sqrt{\frac{\Sigma[N \times n_i^2\,(p_i - p)^2]}{(N-1)\,n^2}}$$

$p_i$ = proportion of larvae surviving discriminating dose at site $i$

$n_i$ = total number of larvae tested at site $i$

N = number of sites

from Sawicki et al. (1989)

## % SURVIVING DISCRIMINATING DOSE

| | | FENVALERATE | | | | | | | | | ENDOSULFAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | | II | | | III | | | I | | | II | | | III | | |
| STUDY AREA | SEASON | av | ± s.e. | n | av | ± s.e. | n | av | ± s.e. | n | av | ± s.e. | n | av | ± s.e. | n | av | ± s.e. | n |
| Namoi/Gwydir | 1983/84 | 9.3 | 1.1 | 1,207 | 9.5 | 1.3 | 842 | 14.6 | 1.8 | 567 | - | - | - | - | - | - | - | - | - |
| | 84/85 | 7.5 | 1.2 | 732 | 12.9 | 1.0 | 2,175 | 27.9 | 1.6 | 2,948 | - | - | - | - | - | - | - | - | - |
| | 85/86 | 7.8 | 0.6 | 1,769 | 13.0 | 0.6 | 4,104 | 44.5 | 1.4 | 5,266 | - | - | - | - | - | - | - | - | - |
| | 86/87 | 32.2 | 1.6 | 1,765 | 36.7 | 1.2 | 3,003 | 42.9 | 1.7 | 4,333 | 7.1 | 1.1 | 895 | 16.7 | 1.5 | 867 | 20.1 | 1.1 | 2,616 |
| | 87/88 | 19.8 | 1.9 | 904 | 30.1 | 1.5 | 1,725 | 38.4 | 1.5 | 2,035 | 7.3 | 1.4 | 229 | 17.6 | 2.2 | 507 | 23.0 | 1.8 | 1,107 |
| | 88/89 | 19.6 | 2.8 | 440 | 42.4 | 3.1 | 434 | 60.7 | 2.0 | 1,055 | 8.8 | 1.8 | 214 | 13.2 | 3.0 | 145 | 10.6 | 1.7 | 667 |
| | 89/90 | 24.7 | 1.6 | 619 | 45.3 | 5.3 | 357 | 62.5 | 2.4 | 690 | 9.2 | 1.9 | 478 | 14.8 | 3.5 | 272 | 15.9 | 1.8 | 589 |
| Emerald | 1985/86 | 6.8 | 0.6 | 7,269 | 17.1 | 1.3 | 2,728 | 14.4 | 0.6 | 5,871 | - | - | - | - | - | - | - | - | - |
| | 86/87 | 8.8 | 0.6 | 2,831 | 26.5 | 1.6 | 1,646 | 29.8 | 1.5 | 3,423 | 7.7 | 1.0 | 1,114 | 20.6 | 1.6 | 1,091 | 17.3 | 1.3 | 2,910 |
| | 87/88 | 15.9 | 1.4 | 2,027 | 27.1 | 2.1 | 838 | 27.0 | 2.1 | 1,975 | 9.5 | 0.9 | 1,036 | 14.3 | 2.3 | 475 | 13.7 | 1.6 | 1,593 |
| | 88/89 | 19.8 | 1.5 | 1,255 | 38.7 | 4.9 | 358 | 44.3 | 1.9 | 1,354 | 8.1 | 1.1 | 1,013 | 13.6 | 3.5 | 259 | 7.1 | 1.1 | 848 |
| | 89/90 | 27.9 | 3.1 | 274 | 44.6 | 7.0 | 77 | 54.6 | 2.3 | 565 | 3.1 | 1.0 | 127 | 21.0 | 5.2 | 42 | 20.9 | 2.6 | 523 |
| Inverell | 1987/88 | 10.2 | 1.8 | 408 | 20.4 | 1.8 | 670 | 19.0 | 1.8 | 481 | 11.3 | 2.1 | 229 | 10.5 | 1.0 | 558 | 5.8 | 1.1 | 292 |
| | 88/89 | 21.9 | 2.8 | 291 | 28.9 | 3.4 | 509 | 41.7 | 2.1 | 720 | 9.4 | 2.4 | 157 | 4.8 | 1.2 | 373 | 5.4 | 1.2 | 615 |
| | 89/90 | 22.1 | 1.8 | 269 | 32.7 | 2.1 | 476 | 38.2 | 6.0 | 97 | 4.0 | 1.3 | 243 | 5.2 | 1.9 | 347 | 7.1 | 5.3 | 78 |

**Table 2.2** Average pyrethroid and endosulfan resistance levels in *Heliothis armigera* for each Stage( I, II & III ) of the Resistance Management Strategy, for three study areas (the Namoi and Gwydir valleys of northern NSW, the Emerald Irrigation Area of central Queensland and a sample of the unsprayed refugia area centred on Inverell in northern NSW). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the discriminating dose(0.2 and 10 micrograms of fenvalerate and endosulfan respectively, per 30-40 mg larva) ± the standard error of the mean. n = the total number of larvae tested in each Stage.

## Impact Of Cropping Pattern On Adult Selection

| Season | Crop Area (ha) | | | Pyrethroid Resistance (Pyr)[a] | | Index of Adult Selection {increase in Pyr between Stages I and II} | Pyrethroid Selection Pressure[b] {sprays / cotton area} |
|---|---|---|---|---|---|---|---|
| | Cotton | Maize (% of total area) | | Stage I | Stage II | | |
| **NAMOI / GWYDIR** | | | | | | | |
| 1983/84 | 49,239 | 150 | (0.3%) | 9.3 | 9.5 | 1.02 ns | 2.3 |
| 1984/85 | 61,242 | 150 | (0.2%) | 7.5 | 12.9 | 1.72 * | 2.1 |
| 1985/86 | 61,709 | 100 | (0.2%) | 7.8 | 13.0 | 1.67 * | 2.7 |
| 1986/87 | 46,533 | 250 | (0.5%) | 32.2 | 36.7 | 1.14 * | 3.2 |
| 1987/88 | 59,221 | 100 | (0.2%) | 19.8 | 30.1 | 1.52 * | 2.6 |
| 1988/89 | 51,091 | 100 | (0.2%) | 19.6 | 42.4 | 2.16 * | 2.3 |
| **EMERALD** | | | | | | | |
| 1985/86 | 8,500 | 2,639 | (24%) | 6.8 | 17.1 | 2.51 * | 0.6 |
| 1986/87 | 6,435 | 1,964 | (23%) | 8.8 | 26.5 | 3.01 * | 2.5 |
| 1987/88 | 11,814 | 602 | (5%) | 15.9 | 27.1 | 1.70 * | 1.7 |
| 1988/89 | 9,000 | 1,000 | (10%) | 19.8 | 38.7 | 1.95 * | 1.5 |

a data from Table 2.2

b data from Figs A3.3 & 5

* , ns indicate Stage II Pyr levels are significantly higher ($p<0.05$) or not significantly different, respectively, from Stage I levels (unpaired t test).

**Table 2.3** Impact of two different cropping regimes (a cotton monoculture in the Namoi and Gwydir river valleys of northern New South Wales and mixed cropping in the Emerald Irrigation Area of central Queensland) on the selection of pyrethroid resistance in adult *Heliothis armigera*.

**Impact of Cotton Price on Stubble Management and the Inter-Season Decline in Pyr — Namoi/Gwydir**

| Winter | % Pyrethroid Resistance (Pyr)[a] | | Index of Inter-Season Decline {decrease in Pyr between previous Stage III and the following Stage I} | % Cotton Stubble left uncultivated[b] | New York Cotton Futures[c] | |
|---|---|---|---|---|---|---|
| | Autumn (Stage III) | Spring / Early Summer (Stage I) | | | Lowest av. monthly proview (US cents/lb) May to August | Cents change between May & Aug |
| 1984 | 14.6 | 7.5 | 1.95** | | 66 | -18 |
| 1985 | 27.9 | 7.8 | 3.58** | | 60 | -5 |
| 1986 | 44.5 | 32.2 | 1.38** | >60 (est.) | 30 | -31 |
| 1987 | 42.9 | 19.8 | 2.17** | 29.1 | 69 | +11 |
| 1988 | 38.4 | 19.6 | 1.96** | 31.2 | 53 | -14 |

a data from Table 2.2

b data from Table 2.6 (1986 data estimate only)

c data derived from Fig 2.10

** indicates Stage I Pyr levels are significantly lower ($p<0.01$) than previous Stage III levels (unpaired t test)

**Table 2.4** Impact of cotton price forecast (for the critical 4 month period after picking) on growers' stubble management decisions and the subsequent effect of these on the survival of the highly resistant overwintering *Heliothis armigera* pupae under cotton in the Namoi and Gwydir river valleys of northern NSW.

**Impact of Refugia in Diluting Pyrethroid Resistance — Namoi/Gwydir**

| Season | % Pyrethroid Resistance (Pyr)[a] | | Index of Total Season Selection Pressure { increase in Pyr between Stages I and III } | Pyrethroid use[b] (sprays / cotton area) | Refugia Dilution Potential | |
|---|---|---|---|---|---|---|
| | Stage I | Stage III | | | Summer rainfall[c] (% of long term mean) | Sorghum production[c] (% of 6 year mean) |
| 1983/84 | 9.3 | 14.6 | 1.57* | 2.3 | +74 | +32 |
| 1984/85 | 7.5 | 27.9 | 3.72** | 2.1 | -57 | -14 |
| 1985/86 | 7.8 | 44.5 | 5.71** | 2.7 | -26 | -23 |
| 1986/87 | 32.2 | 42.9 | 1.33** | 3.2 | -22 | +7 |
| 1987/88 | 19.8 | 38.4 | 1.94** | 2.6 | +11 | +12 |
| 1988/89 | 19.6 | 60.7 | 3.10** | 2.3 | -21 | -13 |

a data from Table 2.2

b data from Fig A3.3

c data from Fig 2.8

* , ** indicate Stage III Pyr levels are significantly higher ($p<0.05$ and $p<0.01$, respectively) than Stage I levels (unpaired t test)

**Table 2.5** Effect of an operational factor (pyrethroid use) and two ecological factors (summer rainfall and sorghum production in the unsprayed Refugia) on the intensity for pyrethroid resistance selection in *Heliothis armigera* from the Namoi and Gwydir river valleys of northern New South Wales.

## Impact of Cotton Price on Stubble Management and Crop Rotation Decisions — Namoi / Gwydir

| Winter | No. Properties (Blocks) surveyed | Crop Residue Class (% of Blocks) | | | | | | | | | | Crop Rotation Class (% of Blocks ) | | | New York Cotton Futures[6] | | Area of[7] cotton (ha) sown the following summer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Little or no cultivation[1] | | | | | | Effective cultivation[2] | | | | | | | | | |
| | | a | b | $c_i$ | $c_{ii}$ | $c_{iii}$ | Total | a | $b_i$ | $b_{ii}$ | $b_{iii}$ | Total | % Cotton[3] after cotton | % Wheat[4] ( or other ) after cotton | % Fallow[5] | Lowest av. monthly proview (US cents/lb) May to August | Cents change between May & August | |
| 1984 | | | | | | | | | | | | | | | | 66 | - 18 | 61,242 |
| 1985 | | | | | | | | | | | | | | | | 60 | - 5 | 61,709 |
| 1986 | estimate* | | | | | | >60 | | | | | <40 | <30 | >40 | >30 | 30 | - 31 | 46,553 |
| 1987 | 98 (535 ) | 3 | 5 | 10 | 9 | 2 | 29 | 31 | 6 | 27 | 7 | 71 | 57 | 26 | 17 | 69 | + 11 | 59,221 |
| 1988 | 87 (583 ) | 2 | 5 | 9 | 13 | 2 | 31 | 41 | 6 | 18 | 4 | 69 | 58 | 29 | 13 | 53 | - 14 | 51,091 |
| 1989 | 94 (563 ) | 1 | 1 | 13 | 16 | 7 | 38 | 42 | 5 | 10 | 5 | 62 | 52 | 34 | 14 | 65 | + 8 | |

* No survey done for 1984 & 85 winters; 1986 survey was a retrospective estimate

1 Little or No Soil Disturbance
- a) Standing stubble, no cultivation
- b) Stalks slashed, no cultivation
- c) Stalks slashed, some cultivation but rows of stalks still intact
  - i) direct drilled with a winter crop
  - ii) aerially sown with a winter crop
  - iii) left fallow

2 Effective Cultivation to Kill Pupae
- a) Existing hills rebuilt for cotton, stalks disturbed
- b) Full cultivation, then
  - i) sown to a winter crop
  - ii) rehilled for cotton
  - iii) left fallow

3,4,5 Crop Residue Classes (2a,2bii ), (1ci, 1cii, 2bi ) & (1a, 1b, 1ciii, 2biii ), respectively

6 Data derived from Fig 2.10

7 Data from Fig A3.1

**Table 2.6** Impact of cotton price forecast (for the critical 4 month period after picking, May to August ) on growers' stubble management and crop rotation decisions for the Namoi & Gwydir river valleys of northern NSW. Survey (part of a collaborative, complementary project with Dr. G. Fitt ) carried out late Sept / early Oct, just prior to sowing and moth emergence from overwintering pupae, to allow the maximum available period for stubble management decisions to be undertaken.

## % Resistance by Host Crop

### FENVALERATE

| Site | Year | Collection Period | | Host | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Stage | Weeks | Cotton | Maize | Sunflowers | Soybean | Sorghum | *V.virgatum* |
| Emerald | 1985/86 | I | 3-4 | | 3.7[a] (701) | 3.4[a] (89) | | | |
| | | | 8-12 | 5.6[a] (823) | 6.6[a] (1,441) | | | | |
| | | II | 1-3 | 15.9[a] (774) | | | 13.9[a] (567) | | |
| | 1986/87 | I | 1-14 | 10.8[a] (148) | 8.1[a] (1,744) | 8.6[a] (441) | | | |
| | | III | 1-3 | 41.2[a] (782) | | | 40.6[a] (367) | | |
| | | | 5-7 | 22.2[a] (54) | 22.8[a] (565) | 23.6[a] (144) | | 16.8[a] (167) | |
| | | | 9-13 | | 21.1[a] (460) | 16.4[a] (269) | | | |
| | 1987/88 | I | 8-10 | 8.6[a] (245) | 8.3[a] (253) | | | | |
| | | III | 8-14 | | | 18.8[a] (536) | | 23.9[a] (209) | |
| | 1988/89 | III | 8-11 | | | 40.8[a] (292) | | 47.2[a] (265) | |
| Namoi / Gwydir | 1986/87 | I | 1-9 | 32.3[a] (655) | 33.0[a] (1,016) | 29.8[a] (94) | | | |
| | 1987/88 | I | 2-5 | 16.4[a] (213) | 19.9[a] (372) | 16.2[a] (99) | | | |
| Inverell | 1987/88 | I | 3-6 | | 10.3[a] (194) | | | | 10.3[a] (175) |
| | | III | 2-9 | | 20.1[a] (294) | 17.5[a] (166) | | | |
| | 1988/89 | I | 1-9 | | 20.3[a] (153) | | | | 17.6[a] (85) |

### ENDOSULFAN

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Emerald | 1986/87 | III | 1-3 | 22.8[a] (639) | | | 26.0[a] (308) | | |
| | | | 5-7 | 16.7[a] (54) | 13.1[a] (528) | 13.0[a] (123) | | 15.2[a] (105) | |
| | | | 9-13 | | 8.0[a] (401) | 5.8[a] (240) | | | |
| | 1988/89 | III | 8-11 | | | 8.3[a] (180) | | 13.7[a] (183) | |
| Inverell | 1987/88 | III | 2-9 | | 7.5[a] (212) | 3.8[a] (80) | | | |

**Table 2.7** Percentage pyrethroid and endosulfan resistance in *Heliothis armigera* larvae reared from eggs collected from various hosts (including the scrophulariaceous weed, *Verbascum virgatum* ). Results expressed as the percentage of larvae, reared on artificial diet, surviving a discriminating dose of fenvalerate or endosulfan (0.2 and 10 µg / 30-40 mg larva, respectively). Numbers in brackets refer to the total number of larvae tested. Collection periods were only analysed where sufficient samples of more than one host, were collected concurrently. Means in the same row, followed by the same letter, are not significantly different ($p<0.05$, chi-squared test).

# CHAPTER 3 - Evaluation of the Impact of the Strategy on Pyrethroid Resistance : $F_1$ Analyses


## Abstract

*The classical resistance monitoring technique using full bioassay lines on laboratory reared F1 progeny of field material was compared to the previously described discriminating dose technique on field collected individuals. The Via tolerance curve analysis of the F1 data clearly indicated the predominance of the oxidative metabolic pyrethroid resistance mechanism from 1984/85 season onwards. There appears to have been an abrupt change in the relative importance of field resistance mechanisms following the introduction of the Resistance Management (IRM) Strategy in 1983/84. The Strategy seems to have favoured the selection of the more amenable oxidative resistance mechanism over the intractable nerve insensitivity mechanism. The Beeman-Nanis analysis was applied to attempt to identify the relative importance of the various field resistance genes. However, it proved of little value in this study as one of the key assumptions underlying the analysis (full genetic dominance) was not satisfied.*


## Introduction

The previous chapter evaluated the impact of the IRM Strategy using a discriminating dose technique on field collected individuals. Although this technique proved extremely successful, its utility was unproven at the commencement of this study. So the classical resistance monitoring technique using full bioassay lines on laboratory reared F1 progeny of field material, was also adopted as a backup study and for comparative purposes. The classical technique was slightly modified by rearing through only survivors of the discriminating dose field screens rather than randomly collected field populations. This modification had been suggested as a possibly more lucrative source of information as far back as 1960 (Davidson) and more recently by Roush & Miller (1986). The main aim of this study was to determine whether the increased workload and cost of the classical F1 bioassay technique yielded any more information (on such things as the relative importance of various resistance mechanisms, etc.) than the simpler, less costly and more sensitive discriminating dose technique.

## Methods & Materials

### *Tolerance Curves*

The sampling procedure, areas and processing were as described in Chapter 2. The fenvalerate discriminating dose survivors for each Stage were reared through to adults, randomly mated and tested as 30-40 mg 3rd or 4th instar larvae in the F1 generation, with either fenvalerate or deltamethrin. At least 90 and up to 500 larvae were tested at each dose within a 0-100% mortality range in each Stage. The total number of larvae tested and the putative number of female parents for each Stage are noted on Figs 3.2-5. Because of the heterogenous nature of these F1 populations, classical probit analysis (Finney 1971) was not considered appropriate. So the data were graphed as

tolerance curves (after Via 1986) with log dose (µg/30-40 mg larva) as the abscissa and incremental kill frequency as the ordinate.

Fenvalerate and deltamethrin tolerance curves for pyrethroid susceptible and homozygote and heterozygote resistant *Heliothis armigera* were also obtained (Fig 3.1). The homozygote resistant colony was pure breeding for a pyrethroid resistance mechanism fully suppressible by piperonyl butoxide (Pbo) (presumably a microsomal monooxygenase) while the heterozygote data were obtained by pooling the results for the reciprocal crosses between males and females of the homozygote resistant and the susceptible strain. Because heterozygotes usually dominate in the early stages of a resistance episode, the heterozygote tolerance curve was chosen for comparison with the actual recorded field data. Thus the fenvalerate or deltamethrin heterozygote tolerance curves from Fig 3.1 were superimposed on each tolerance curve in Figs 3.2-5.

Fenvalerate was tested on Namoi/Gwydir material from the inception of the Strategy in 1983/84 season until Stage 1 in the 1986/87 season. Fenvalerate testing at Emerald did not begin until the third year of the Strategy (1985/86 season) and finished at the same time as the Namoi/Gwydir. Deltamethrin testing was similar except it was terminated at the end of the 1985/86 season in both areas.

The individual data for each Stage were combined for each season in each area, Figs 3.2-4. (Note, because of low numbers, only the combined Stages data are given for 1983/84 season). These were further combined for all Stages/all seasons in each area and then still further to all Stages/all seasons/both areas combined, Fig 3.5.

### *Beeman-Nanis Analysis*

In 1986, Beeman & Nanis presented an analysis for comparison of discriminating dose testing of field material and F1 survivors. Their main aim was to check if one particular gene controlled most or all field resistance in their area of interest (malathion resistance in *Tribolium castaneum*). This had been suggested by their laboratory studies and they wanted to extract further information from their resistance testing to verify this. This was analagous to this study which also aimed to attempt to extract further information from the monitoring programme, such as the relative importance of various resistance mechanisms. Beeman & Nanis compared the frequency of susceptibles in field strains of *T. castaneum* with the following generation after selection at a dose that killed all susceptible individuals. This information can be easily extracted from the data in this study. The responses of 10 Namoi/Gwydir and 4 Emerald field populations of *H. armigera*, to one generation of selection with a discriminating dose of fenvalerate (0.2 µg/30-40 mg larva), is given in Fig 3.6. Each data point represents the overall average for each Stage of the Resistance Management Strategy from Stage 1, 1983/84 to Stage 1, 1986/87 in the Namoi/Gwydir and Stage 1, 1985/86 to Stage 1, 1986/87 at Emerald. The parental generation data come from Table 2.2 and the F1 generation data were derived from Figs 3.2 & 3. The predicted response line in Fig 3.6 is the theoretical response that would occur in an ideal population after one generation of premating selection at a discriminating dose for a dominant resistant allele in Hardy-Weinburg equilibrium. If $p$ equals the frequency of the susceptible(s) allele, then the frequency of genotypically susceptible (f s/s) larvae in the parental

population is $p^2$ (abscissa in Fig 3.6) at H-W equilibrium. After one generation of selection the theoretical frequency of susceptible larvae can be shown to be $(p/p+1)^2$ (ordinate in Fig 3.6). Beeman & Nanis' analysis is subject to three important assumptions :-

1) premating selection at a single diallelic locus in H-W equilibrium
2) alleles of equal fitness in the absence of insecticide
3) dominant resistance allele

## Results

### *Tolerance Curves*

Both the fenvalerate and deltamethrin susceptible tolerance curves overlapped to some degree the heterozygote and homozygote resistant tolerance curves (Fig 3.1). This has been also noted by Daly (1988) and Daly & Murray (1988). The heterozygote tolerance curves for both pyrethroids were intermediate between the susceptible and homozygote curves, indicating a semi-dominant gene controlling this resistance mechanism (oxidative metabolic detoxification). This has also been found by Daly (1988) and Gunning & Easton (1987). The tolerance curves in Fig 3.1 all approximated fairly well the expected bell shaped normal population type curve except for the susceptible deltamethrin population. This was also noted in Appendix 2 which indicated a greater variability in $LD_{50}$s and lower slopes for deltamethrin in comparison to other pyrethroids.

The fenvalerate tolerance curves in the Namoi/Gwydir and Emerald (Figs 3.2 & 3.3, respectively), all indicated a great deal of heterogeneity in the F1 populations. However, one common factor was a significant component of their populations which matched quite well the heterozyzote tolerance curve. Peaks and plateaus to the left of the heterozygote tolerance curve (around 0.03 µg/larva, from Fig 3.1) indicate the presence of susceptibles, as would be expected in the F1. Peaks and plateaus to the right of the heterozygote tolerance curve (around 2.0 µg/larva, from Fig 3.1) indicate the presence of homozygotes, also expected to be present in the F1 . Interestingly, small variable peaks were detected still further to the right (between 10 & 100 µg/larva), particularly in the Namoi/Gwydir 1983/84 season. In fact this particular season was quite anomalous in comparison to later years. There were two distinct populations present; a susceptible and a highly resistant population which correlated poorly with either the heterozygote or homozygote oxidative resistance tolerance curves. (N.B. It should be noted for later discussion, that the Emerald site was not investigated until 2 years later, i.e. 1985/86 season).

The deltamethrin tolerance curves in the Namoi/Gwydir and Emerald (Fig 3.4) indicate a similar situation as for fenvalerate. The heterozygote tolerance curve matched a significant component of the population. The bimodal susceptible tolerance curve (0.005 to 0.05 µg/larva, from Fig 3.1) could be detected quite distinctly but the homozygotes (at about 0.3 µg/larva, from Fig 3.1) were more difficult to discern. As for fenvalerate, small highly resistant populations could be detected (between 0.5 & 5 µg/larva), once again particularly in the anomalous 1983/84 season.

*Beeman-Nanis Analysis*

The data correlated extremely poorly with the predicted theoretical response (Fig 3.6), consistently and significantly overestimating the proportion of susceptibles in the F1.

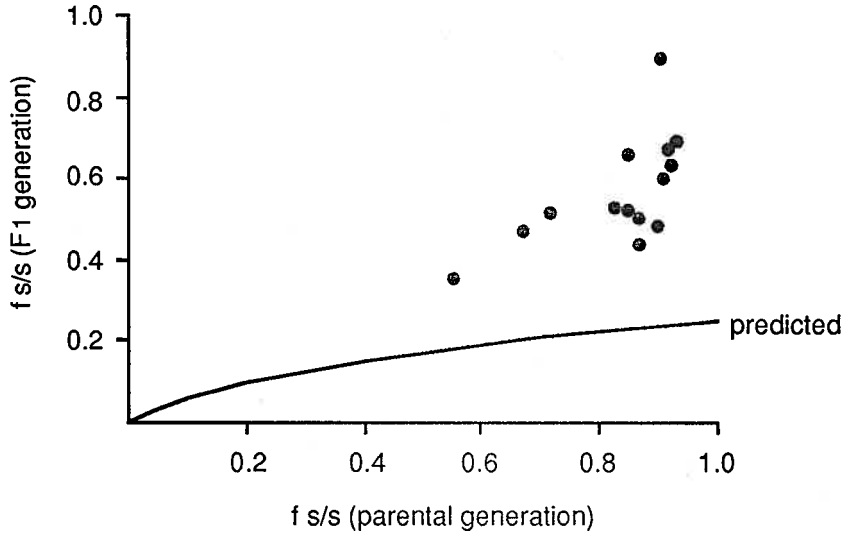


**Fig 3.6** Beeman-Nanis analysis of F 1 data (see text for details).

## Discussion

The F1 tolerance curves indicated clearly the dominance of the oxidative metabolic resistance mechanism, at least from the 1984/85 season onwards. The anomalous 1983/84 season is quite interesting as it indicates a possible major shift in pyrethroid resistance mechanisms since the introduction on the Resistance Management Strategy. The very early resistance mechanism/s gave very high orders of resistance and correlated poorly with the expected oxidative metabolic resistance population response. These early populations had been subject to continued, intense and unrestrained selection pressure for some years, which had resulted in the first field failures occurring late in the previous 1982/83 season. Thus the 1983/84 population (first year of the IRM Strategy) would have still reflected pre-Strategy selection pressure to a degree and may have expressed elevated levels of Pbo insensitive resistance mechanisms, such as *kdr* or even super *kdr* type nerve insensitivity. This scenario has also been suggested by Gunning et al. (in press) who arrived at the same conclusion by different means. The possibility that the IRM Strategy has favoured the selection of the oxidative resistance mechanism over the nerve insensitivity mechanism is not only academically intriguing but is also extremely important at the practical level. The more amenable oxidative resistance mechanism can be challenged by synergists and metabolically refractory altered pyrethroid structures. However, there are no known means to overcome the highly intractable nerve insensitivity mechanisms. These issues are discussed more fully in Chapters 8-10.

The Beeman-Nanis analysis also endeavoured to indicate the presence of a single predominant resistance mechanism. In their 1986 paper, the three key assumptions for the validity of the analysis were met and their actual and predicted response curves were highly correlated. They

concluded that their field resistance was due to a single dominant gene. However, the analysis is less useful for this study as the third key assumption (genetic dominance) was not satisfied. A semi dominant allele (indicated earlier), which allows overlap of the susceptible and heterozygote lines, will underestimate resistance and therefore overestimate susceptibility in the F1 generation. This explanation could well explain the failure of the Beeman-Nanis analysis in this case. However, the presence of multiple resistance genes or reduced fitness (assumptions 1 and 2), cannot be ruled out as contributing to the failure, although the latter seems unlikely (see Chapter 5). The Beeman-Nanis analysis will probably only be of use in identifying the relative importance of resistance genes in the field, where they are fully dominant.

The Via tolerance curve analysis of the F1 data did allow extra information to be gleaned, compared to the single discriminating dose approach. This latter technique had not been designed to detect possible changes in the relative importance of various resistance genes. The former technique however, did indicate these changes, albeit somewhat subjectively. However, the tremendous workload and cost of the F1 technique was becoming prohibitive, so it was decided to modify the discriminating dose technique, to generate the same information but more economically and more precisely. This concept forms the basis of a "dual" insecticide/synergist discriminating dose technique which was introduced in the 1987/88 season and described fully in Chapter 8.

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## PYRETHROID TOLERANCE CURVES : SUSCEPTIBLE & RESISTANT *Heliothis armigera*

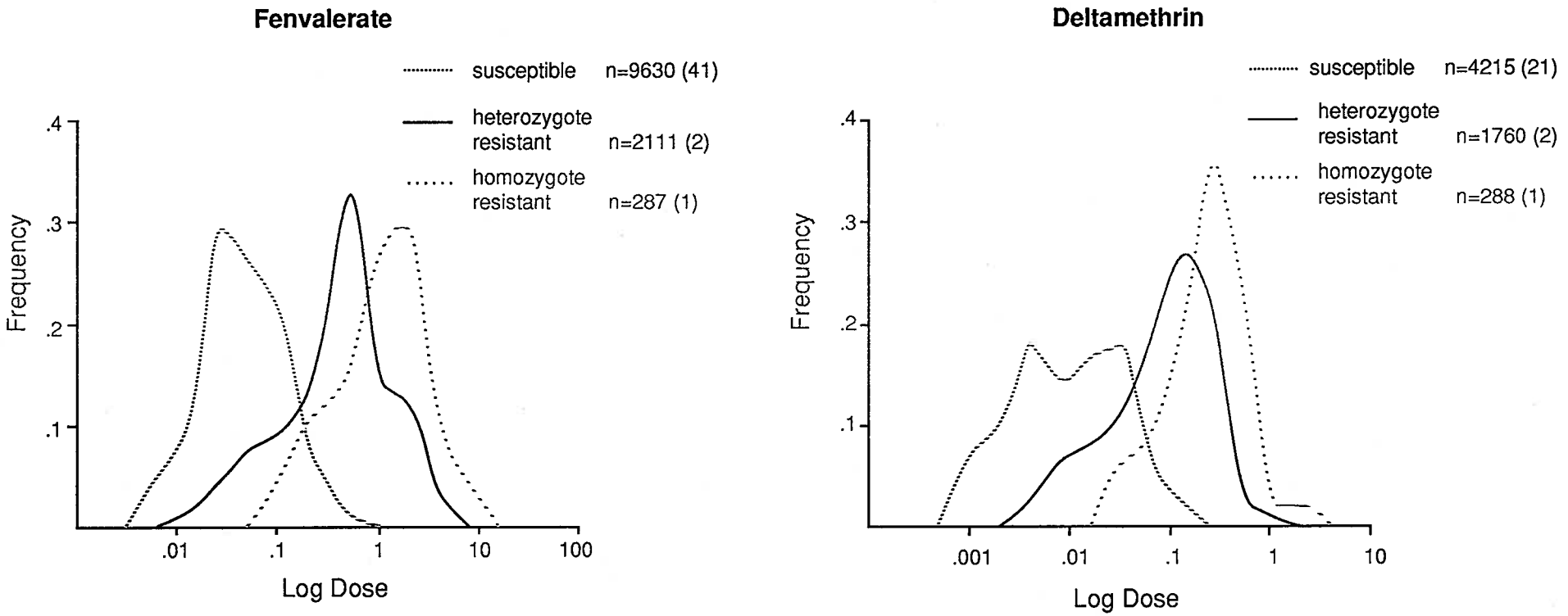


**Fig 3.1** Tolerance curves (after Via 1986) for the fenvalerate and deltamethrin bioassay of susceptible and pyrethroid resistant *Heliothis armigera*. Abscissa - log dose (µg/30-40mg larva). Ordinate - incremental kill frequency for that dose. Homozygote resistant colony pure breeding for a pyrethroid resistance mechanism fully suppressible by piperonyl butoxide (presumably a microsomal monooxygenase). Heterozygote data pooled for the reciprocal crosses between males and females of the homozygote resistant and a susceptible strain. n = total number of larvae tested. Number of colonies tested in brackets.

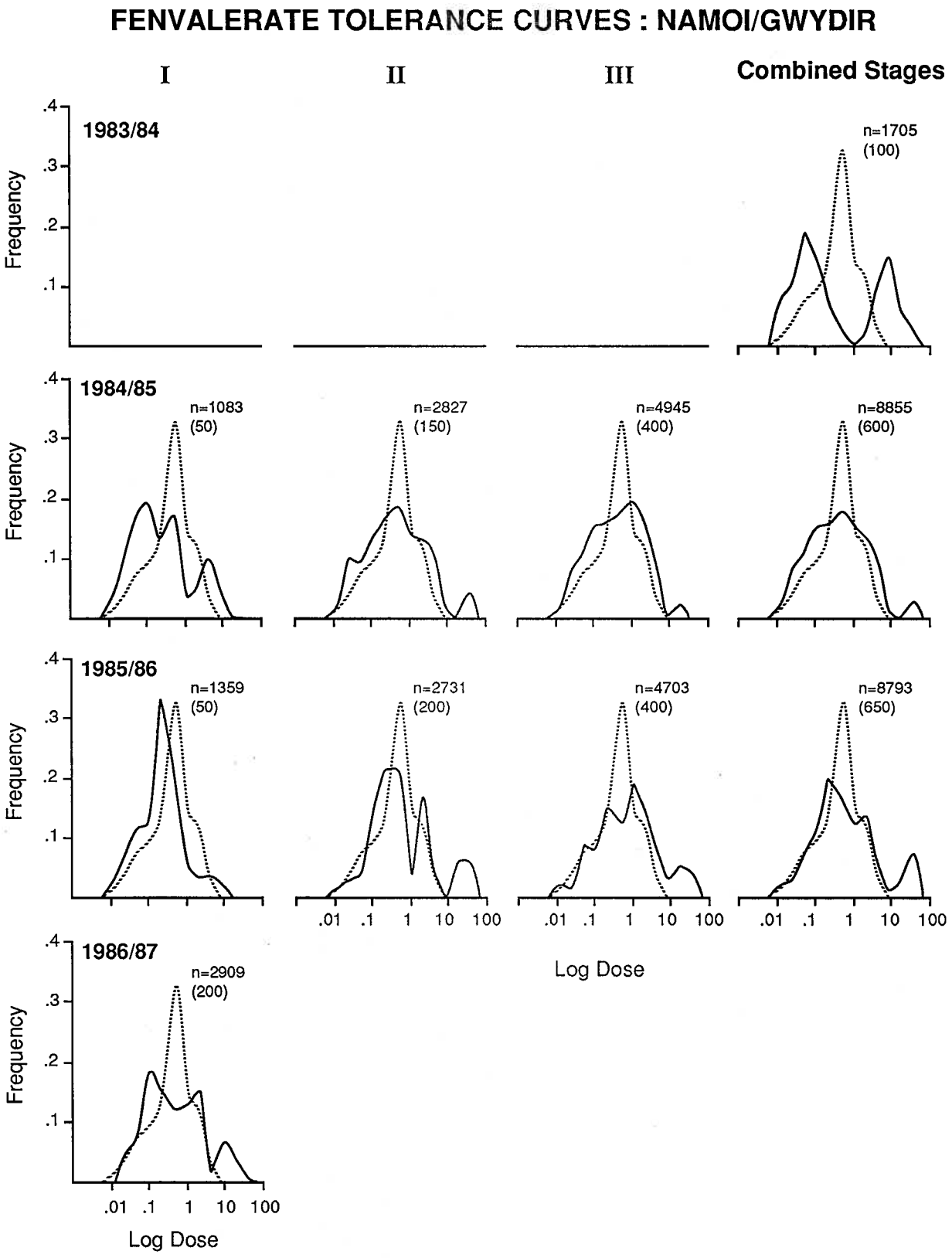


**Fig 3.2** Tolerance curves (after Via 1986) for the fenvalerate bioassay of F1 progeny of *Heliothis armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir study area (1983/84 to 1986/87). Abscissa - log dose (µg/30-40 mg larva). Ordinate - incremental kill frequency for that dose. Combined = Stages I, II, III together. n = total number of larvae tested. Number of putative female parents in brackets. Dotted background figure is the tolerance curve for the fenvalerate bioassay of a strain heterozygous for a resistance mechanism fully suppressible by piperonyl butoxide (presumably a microsomal monooxygenase), from Figure 3.1.

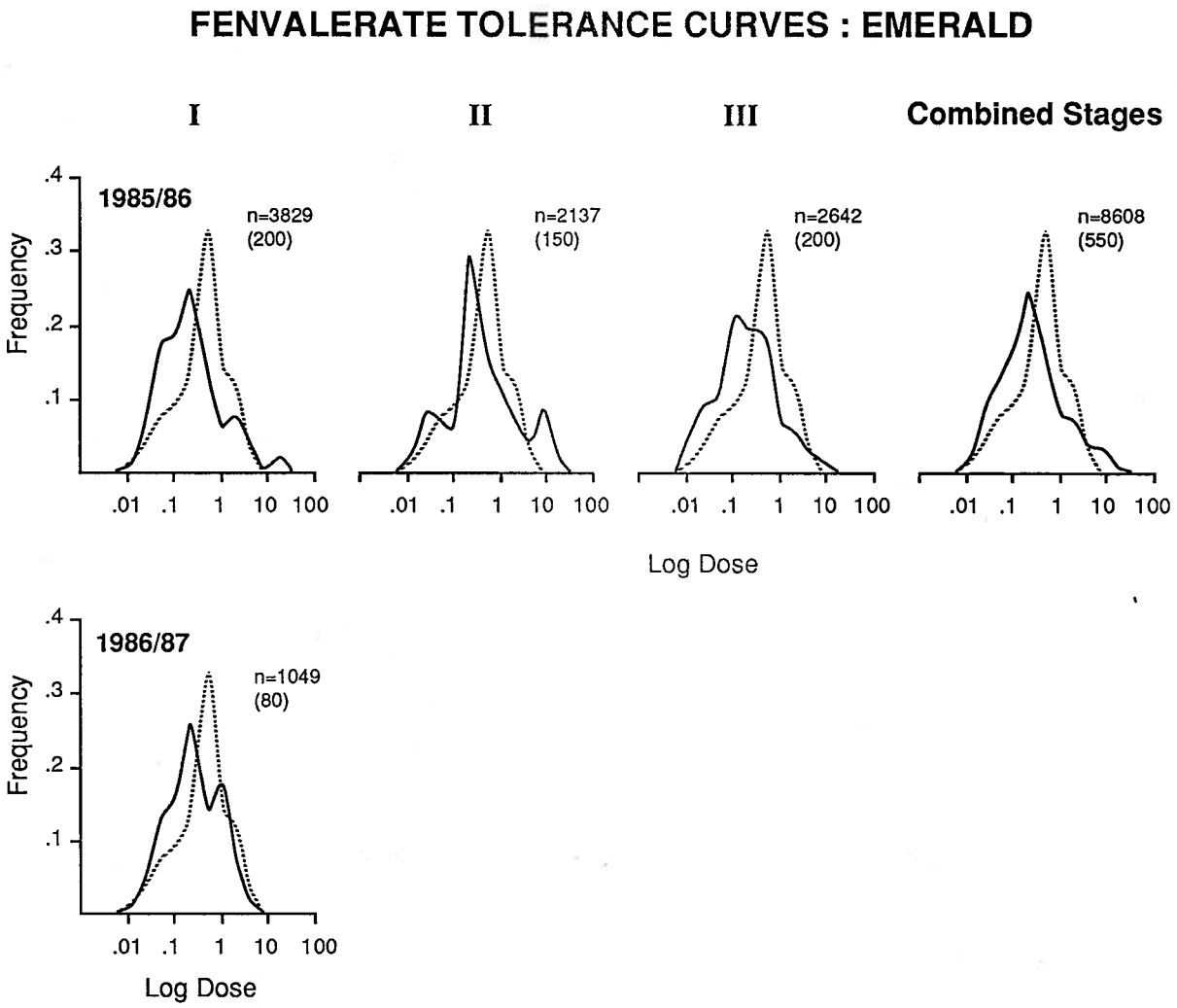


**Fig 3.3** Tolerance curves (after Via 1986) for the fenvalerate bioassay of F1 progeny of *Heliothis armigera* fenvalerate discriminating dose survivors from the Emerald study area (1985/86 to 1986/87). Abscissa - log dose (µg/30-40 mg larva). Ordinate - incremental kill frequency for that dose. Combined = Stages I, II, III together. n = total number of larvae tested. Number of putative female parents in brackets. Dotted background figure is the tolerance curve for the fenvalerate bioassay of a strain heterozygous for a resistance mechanism fully suppressible by piperonyl butoxide (presumably a microsomal monooxygenase), from Figure 3.1.

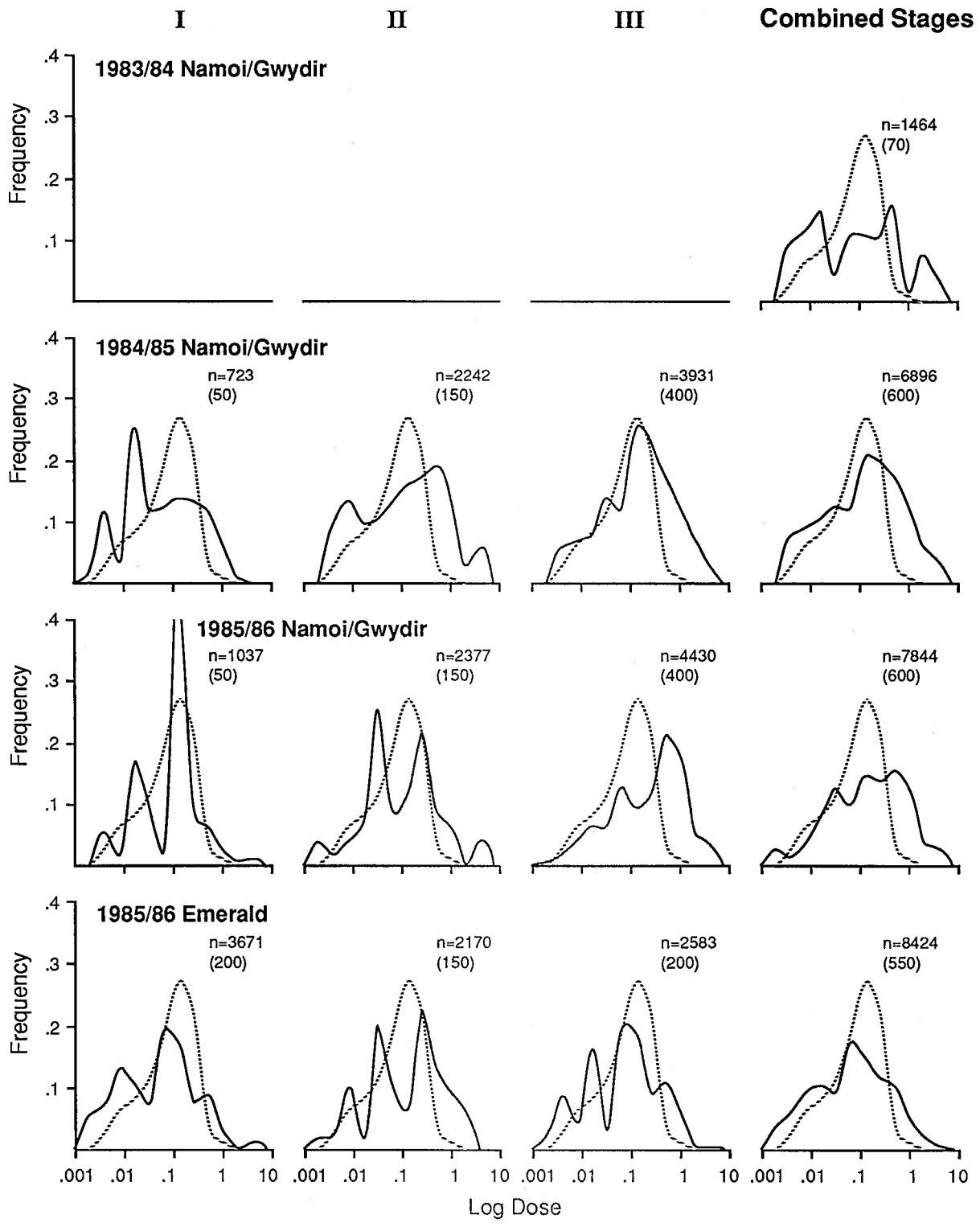


**Fig 3.4** Tolerance curves (after Via 1986) for the deltamethrin bioassay of F1 progeny of *Heliothis armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir (1983/84 to 1985/86) and Emerald (1985/86) study areas. Abscissa - log dose (µg/30-40 mg larva). Ordinate - incremental kill frequency for that dose. Combined = Stages I, II, III together. n = total number of larvae tested. Number of putative female parents in brackets. Dotted background figure is the tolerance curve for the deltamethrin bioassay of a strain heterozygous for a resistance mechanism fully suppressible by piperonyl butoxide (presumably a microsomal monooxygenase), from Figure 3.1.

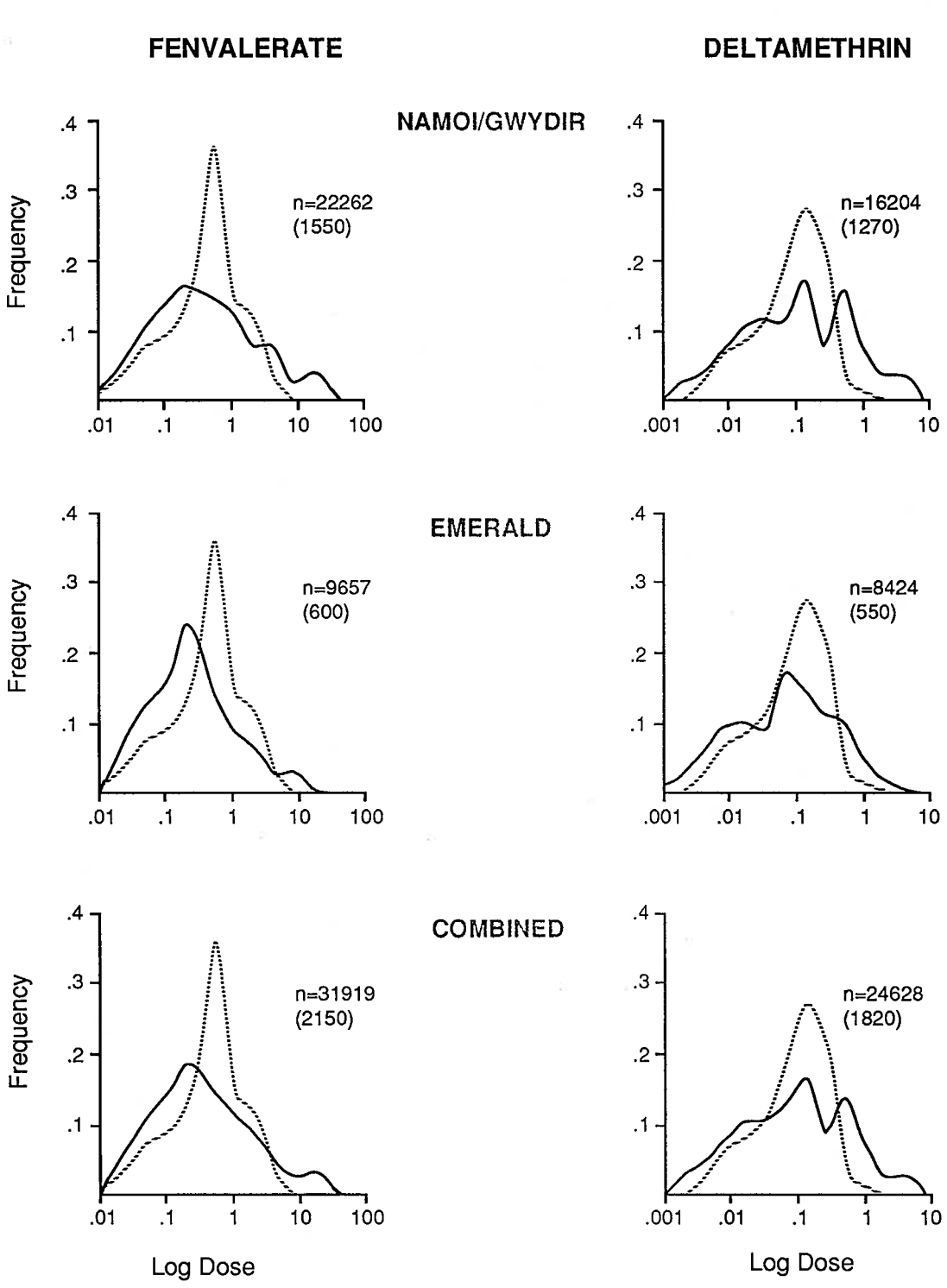


**Fig 3.5** Tolerance curves (after Via 1986) for the fenvalerate and deltamethrin bioassays of F1 progeny of *Heliothis armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir and Emerald study areas, singly and combined. Data from all stages and all years (1983/84 to 1986/87) pooled. Abscissa - log dose (µg/30-40 mg larva). Ordinate - incremental kill frequency for that dose. n = total number of larvae tested. Number of putative female parents in brackets. Dotted background figures are the tolerance curves for the respective fenvalerate and deltamethrin bioassays of a strain heterozygous for a resistance mechanism fully suppressible by piperonyl butoxide ( presumably a microsomal monooxygenase), from Figure 3.1.

# APPENDIX 1 - Rearing Methods for *Heliothis* spp.

## *Moths*

On emergence, moths are placed into 27 litre ventilated perspex mating cages. Up to 20-30 pairs are placed in each cage. The moths are fed a 10% honey solution which is changed 3 times a week. Honey feeders are of two types: 4.5 ml drop dispensers hung from the top of the cage (4 per cage) and 28 ml containers stuffed with cotton wool, soaked in honey solution and placed on the cage floor (3 per cage). 0.1% oxytetracycline hydrochloride is added to the honey solution to control possible bacterial contamination. Paper towel is used to line the bottom of the cage whilst fine white muslin is used on the sides. Moths lay mostly on the muslin liners. Eggs are harvested 3 times a week, washed off in 0.1% sodium hypochlorite solution and collected by filtering the suspension through a Buchner funnel. The concentrated eggs are then rinsed by flushing with water and left to dry until the filter paper is just moist to the touch. The filter paper is then clipped to a sheet of paper towelling which is placed inside a 250 x 305 mm plastic bag and sealed with masking tape. The bag is hung vertically in a constant temperature room (25 ±1° C) with the tape seal to the bottom. As the neonates are negatively geotropic, this minimises larval losses due to escapes or entrapment on the tape. The moth rearing room is maintained at 25 ± 2° C and 14 hours light/10 hrs dark. Humidity is maintained at 70-80% for the last 2 hours of the day cycle and the entire 10 hr night cycle, using a steam humidifier.

## *Small Larvae*

Neonates are transferred with a very soft, fine hair paintbrush onto artificial diet (Table A1.1) in 11cm dia (300 ml) round plastic tubs. A 7 cm diameter hole in the lids of these containers is covered with a semi - permeable plastic wrap (Rapfast, Kent Paper Co., Spit Junction, N.S.W.) to allow adequate aeration without loss of larvae. About 30 neonates are placed in each container (stacked with the lids down to minimise escapes) and when they reach the second instar are separated to prevent carnibalism. Larvae are transferred with soft blunt nosed forceps to individual wells of 12 well larval rearing trays. These trays are Linbro tissue culture trays (Flow Laboratories, North Ryde, N.S.W.) modified for entomological use by removal of the small aeration lugs to allow better sealing. The use of these trays has resulted in substantial savings in labour and operating costs as the trays allow handling of 12 larvae with the one lid on/off operation. They are also stackable and therefore spacesaving, sturdy enough to be recycled and transparent to allow efficient viewing of larval development without lid removal. They also save on diet as only 2-3 mls of diet is poured into each of the 7.5 ml (2.4 cm dia) wells.

The diet is poured into the trays from modified soft plastic squeeze bottles (outlet tube cut short and internal tube removed). Generally, 100 trays can be poured from one batch of diet, kept warm and pourable by immersing the blender jug containing the diet, into a hot water bath. Diet is poured inside a laminar flow cabinet and allowed to cool there. This procedure has virtually eliminated the

incidence of fungal contamination of diet by *Aspergillus* spp. etc. Once cool, trays of diet are kept in the refrigerator (not frozen) until required.

ARTIFICIAL DIET RECIPE

| | |
|---|---|
| Blend up | — 1 litre hot water |
| | — 60 gms wheat germ |
| | — 53 gms brewer's yeast |
| | — 130 gms soybean flour |
| | — 3.3 gms nipagin |
| | — 1.7 gms sorbic acid |
| | — 13.5 mls of 10% formaldehyde |
| | — 7 mls pure sunflower oil (no antioxidants) |
| Combine with | — 16 gms agar |
| | — 300 mls boiling water |

Blend, allow to cool and add 5.3 gms of ascorbic acid when diet reaches less than 60° C.

Table A1.1 Artificial diet for larval *Heliothis* spp., based on a modification of Teakle & Jensen (1985).

### *Large Larvae*

Larvae for bioassay are sorted and tested daily in fresh trays. Larvae being reared to the adult stage are left in the 12 well trays until the last instar when they are placed on 8-10 cc of artificial diet in 28 ml Rheem plastic pots (Durapak, Lidcombe, N.S.W.). This diet is slightly modified from the early instar/testing diet in that the amount of agar is increased by 50% and 2.2. g of oxytetracycline hydrochloride is added to control bacterial infections. The extra agar is added to reduce the moisture content of the final instar diet/prepupal burrowing medium which was found necessary to improve larval to pupal viability. Contaminant yeasts/bacteria can cause fermentation of the diet at this stage, particularly on diet older than 7 days. This problem can be overcome by adding 1.2 g of sodium metabisulphite or 50 mg chloramphenicol to the diet.

The pots housing the last instar larvae are kept in place by perspex frames and lids, primarily to prevent escape of large larvae from chewing through the soft plastic lids supplied with the pots. These frames are stackable, reusable and hold 48 larvae under one lid. The size and number of holes in the lid above each pot is critical to allow a fine balance between the need to dry the diet for optimum pupation conditions and the need for the diet to remain moist enough to be palatable (four 2

mm dia holes/pot have been found to be ideal). The base frame and lid are held together by five 30 mm long, 4 mm dia. bolts with wing nuts for easy removal.

### *Pupae*

Larvae pupate in the residual diet medium and when the pupal case has hardened, pupae are removed with soft, blunt nosed forceps and surface sterilised in 0.1% sodium hypochlorite for 8 minutes. Pupae are then dried, sexed and placed in dry vermiculite in 1.2 litre ventilated plastic emergence jars. Up to 30 pupae are placed in each jar and paper towelling is placed around one side of the emergence jars to enable a suitable vertical surface for newly emerged moths to expand their wings.

### *Tray Recycling*

Larval rearing trays are cleaned with a high pressure water blaster. Trays are held in place by a specially designed metal frame and old diet, frass and dead larvae are removed quickly and efficiently by the sprayer. Any remaining residues are removed by hand washing. Trays are sterilised before reuse by soaking in 0.2% sodium hypochlorite for at least 6 hours. They are then rinsed in fresh water and dried in direct sunlight.

### *Species Suitability*

The rearing methods described have been found to be equally effective for both *Heliothis armigera* and *Heliothis punctigera.*

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# APPENDIX 2 - Base Line Susceptibility Data for *Heliothis* spp. and Calibration of Discriminating Doses

## Introduction

The basis for any resistance monitoring programme requires an accurate knowledge of the susceptible phenotypic response. Consequently, it is important to isolate and establish susceptible colonies to measure the organism's base line response level. Ideally this should be done before any resistance development (Tabashnik 1986), otherwise it can become quite difficult to find and establish susceptible strains. In this study, a number of susceptible strains were collected off a range of hosts from a wide geographical area and their bioassay response checked against a standard laboratory susceptible strain.

Historically, resistance has been most popularly measured by changes in log dose probit (ldp) responses but discriminating doses had been used also (eg. Georghiou & Taylor 1976). However, even as far back as 1960, Davidson had recognised the importance of using discriminating dose testing when measuring resistance in heterogenous populations. The decision to use discriminating doses from the beginning of this study (1983/84 season) was subsequently vindicated by Roush & Miller's (1986) illuminating analysis indicating that "a discriminating dose test is more efficient than a dose/response regression in monitoring for resistance." However, ldp lines are also very useful for certain toxicological studies (especially cross resistance studies), so both base line bioassays and discriminating doses were calibrated on various susceptible strains, for the insecticides of interest in this study.

## Methods & Materials

### *Insecticides*

Various pyrethroid and cyclodiene insecticides were made up as solutions of technical material dissolved in analytical grade acetone. Sources of insecticides were:- fenvalerate 94.3% a.i. from Shell, Melbourne; deltamethrin 99.4% a.i. from Hoechst, Melbourne; cypermethrin 93.7% a.i. from FMC, Brisbane; endosulfan 98.1% a.i. from Hoechst, Melbourne; dieldrin 99.0 % a.i. from Shell, Melbourne; endrin 96.0% a.i. from Velsicol, Sydney and Resistance Breaking Pyrethroid 86.0% a.i. from I.C.I., U.K.

### *Source of Colonies*

Various strains of susceptible *Heliothis armigera* and *Heliothis punctigera* were collected as eggs or larvae on a range of host crops from 1983 to 1985 and tested in the 1st to 3rd laboratory generation. Long term susceptible laboratory cultures of both species, obtained from Dr. R. Teakle, Department of Primary Industries, Brisbane, Qld, were used as checks for susceptibility of the field collected strains. The long term laboratory culture of pyrethroid and endosulfan susceptible *H.armigera*, collected originally off sorghum at Gatton, Qld in 1979, was also tested on a number of occasions after the 34th generation to determine:- a) the repeatability of bioassay results an a standard colony and b) the effect of long term laboratory culture on phenotypic expression of susceptibility.

### *Larval testing*

Larvae were reared on artificial diet (see Appendix 1), checked twice daily and 3rd or 4th instars weighing 30-40 mg were placed on fresh diet and tested with various pyrethroid and cyclodiene insecticides. Each larva was dosed dorsally on the thorax with 1 µl of insecticide /acetone solution with a Hamilton 50 µl microsyringe in a repeating dispenser (Alltech Associates, Homebush, N.S.W.). A calibration test was also caried out for fenvalerate on 40-60 mg *H.armigera* larvae. Larval mortality was assessed at 3 days post treatment (held at 25 ± 2°C). Larvae were considered dead if they were unable to move in a co-ordinated manner, when prodded from behind.

### *Moths : Topical Eye Test*

Male and female moths were kept separate on emergence and fed for 1 day on 10% honey solution. They were then weighed, anaesthetised briefly with carbon dioxide and dosed on the left eye with 1 µl of insecticide/acetone solution. Moths were held individually in 28 ml containers for 24 hrs after treatment, to assess the initial response. Moths showing any signs of activity were then held in 300 ml plastic tubs (in groups of 2-5) with access to 10% honey, for a further 48 hours, to allow for any recovery. Final moth mortality was assessed at 3 days post treatment (held at 25 ± 2°C). Moths were considered dead if they were unable to move (walk or fly) in a co-ordinated manner, when prodded from behind. Because of variation in moth weights (both within and between sexes) $LD_{50}$'s were adjusted to a standard 200mg moth.

### *Moths : Tarsal Plate Test*

Male and female moths were treated as above for the first day. After weighing, they were quickly placed in groups of 2-5 on a treated glass petri dish bottom (9 cm diameter, 1 cm deep). The petri dish bottoms had been treated with 1 ml of insecticide/acetone solution which was just enough to cover the surface of the plate with a thin film. The acetone was allowed to evaporate off and the insecticide dose was expressed in µg of toxicant/$cm^2$ of glass surface. After the moths had been placed on the treated plates, an untreated lid was placed on top in order to confine the moths to walking on the treated surface. Moths were held on the plates for 24 hours and then those showing any signs of activity were held as above, for a further 48 hours. Mortality was assessed at 3 days post treatment as above and $LC_{50}$'s were also adjusted to a standard 200 mg moth.

### *Statistical Analysis*

At least 48 larvae and 36 moths were tested at each dose within a 0-100% mortality range. Any control mortality was corrected with Abbott's formula. Log dose probit lines were analysed using the Genstat statistical package.

## Results

### *Larval Heliothis armigera : Pyrethroids / Cyclodienes*

Fenvalerate was tested on 37 susceptible strains for 30-40 mg larvae (Table A2.1). Considering the wide variation in collection sites and hosts (Melbourne in the south to Darwin in the

north), there was remarkably little variation (2.5 fold) in the LD50 response (0.02-0.05 µg/larva). Slopes were generally quite high (average 3.0) which resulted in a confident determination of a discriminating dose (0.2 µg fenvalerate averaged out at 99.1 ± 0.3% kill for 30-40 mg larvae). However, this dose was shown to be too low on the larger 40-60 mg larvae and 0.5 µg fenvalerate proved to be a better discriminating dose (99.7 ± 0.3% kill).

Deltamethrin was assessed on 21 susceptible strains over a similar range of collection sites and hosts as for fenvalerate (Table A2.2). There was more variability (4.0 fold) in the response to deltamethrin (LD50 range 0.004-0.016 µg/larva) and much lower slopes (average 1.7). Because of these low slopes and greater variability, no attempt was made to calibrate a discriminating dose.

Endosulfan was assessed on 8 susceptible strains (Table A2.3). There was little variability (2.0 fold) in response to endosulfan (LD50 range 0.49-0.98 µg/larva) and reasonably high slopes (average 2.6) which resulted in a confident determination of a discriminating dose (10µg endosulfan averaged out at 98.3 ± 1.2% kill for 30-40 mg larvae. As endrin and dieldrin were only being used in cross resistance studies, they were tested only on the laboratory susceptible colony and no discriminating doses were evaluated.

The repeated bioassays on the laboratory susceptible colony indicated excellent consistency of results for all 3 of the compounds tested, over a 7 year testing period (Table A2.4).

The Resistance Breaking Pyrethroid (RBP) was not available for testing until late 1989, so it was only able to be calibrated on the laboratory susceptible colony (Table A2.5). Repeated bioassays on this strain indicated little variability (2.0 fold) in response to RBP (LD50 range 0.015-0.031 µg/larva) and generally high slopes (average 3.1). A large number of larvae were screened at a number of candidate doses, with 0.1 µg/30-40mg larva being chosen as the most suitable discriminating dose (96.7% kill).

### *Larval Heliothis punctigera : Fenvalerate / Endosulfan*

Fenvalerate and endosulfan were assessed on 10 and 11 susceptible strains, respectively from a wide range of collection sites and hosts (Table A2.6). There was little variation (2-2.5 fold) in the LD50 response for either chemical and slopes for both were high (3.6 and 3.5 average, respectively) which resulted in a confident determination of discriminating doses (99.1 ± 0.4% and 96.0 ± 1.3% kill on 30-40 mg larvae for 0.05 µg fenvalerate and 2.5 µg endosulfan, respectively).

### *Adult Heliothis armigera : Pyrethroids / Cyclodienes*

Fenvalerate was assessed using both testing techniques on repeated bioassays of the laboratory susceptible colony (2 and 3 assays for the topical eye and tarsal plate tests, respectively) (Table A2.7). With both testing techniques, there was no significant differences in bioassay responses between the sexes and there was excellent consistency of assay results over time (weights adjusted to standard 200 mg moth). Candidate doses were evaluated for both techniques and 2.0 µg/moth (topical eye test) and 1.57 µg/cm$^2$ (tarsal plate test) were chosen as the most suitable discriminating doses, giving 100% and 98.3% kill, respectively.

Cypermethrin/RBP and cyclodiene results still to be finalised.

## Discussion

The fenvalerate and deltamethrin LD50's (µg/larvae) & slopes for *H. armigera* obtained in this study (0.03/3.0 & 0.009/1.7, respectively) agree well with previously published data (0.03/3.0 & 0.010/2.6, respectively) from Gunning et al. (1984). Except for deltamethrim, there was relatively little variation in the LD50's (less than 2.5 and 2.0 fold, for fenvalerate & endosulfan, respectively). This variation is slightly lower than the 3-7 fold variation found for similar data for aphids and diamondback moth (Stribley et al. 1983, Furk & Roberts 1985, Zoebellein 1986). It is also very much lower than the 12 fold variation found for susceptible laboratory cultures of *Heliothis virescens* in the USA (Staetz 1985). It was suggested that this variation was due to long term cultures becoming inbred, less vigorous and usually more susceptible than the original population from which they were taken (Staetz 1985). If this problem was universal, then it would be risky to rely on long term susceptible laboratory cultures which could overestimate the degree of resistance. However, in this study, no evidence of increasing susceptibility could be found in the long term laboratory "Teakle" strain of susceptible *H. armigera* for any of the compounds tested. So this strain, at least during the course of this study, remained a suitable benchmark for susceptibility.

The greater variability and lower slopes for deltamethrin bioassays in comparison to other pyrethroids, has also been noted previously. Gunning et al. (1984) noted this for deltamethrin versus fenvalerate in susceptible *H. armigera* and Leonard et al. (1988) for deltamethrin versus fenvalerate and cypermethrin in susceptible *H. virescens* and *H. zea*. Thus, deltamethrin was not considered suitable for confident determination of a discriminating dose with narrow error estimates (Sawicki et al.1989, Halliday & Burnham 1990), despite it being the most popular pyrethroid in commercial use at the time (see Appendix 3). Because of its higher slope, fenvalerate (also used commercially at the time), was considered better for this purpose.

Both adult testing techniques gave consistent results and high slopes. Topical testing of adult Lepidoptera has always been a problem in the past due to the need to remove scales to allow accurate dosing. In order to avoid this problem, dosing on the eye was evaluated as this was the most accessible scale free cuticle available. Welling & Paterson (1985) suggested that the compound eye cuticle, even though without pore canals, could still manifest substantial absorption of insecticides. The technique proved very simple and effective, giving results as good as the more commonly used residue on glass technique. This latter self dosing technique needs careful design to minimise problems of avoidance behaviour (Brown & Brogdon 1987), particularly with the strongly repellent pyrethroids.

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## Bioassay of Susceptible Larval *Heliothis armigera* : FENVALERATE

### 30 - 40 MG LARVAE

| Strain Data | | LD50 | 95% Conf Interval | | Slope | % Kill @ µg/larva | | |
|---|---|---|---|---|---|---|---|---|
| Collection Site | Host | (µg/larva) | Lower | Upper | | 0.1 | 0.2 | 0.5 |
| Narrabri, NSW | cotton | 0.02 | 0.021 | 0.029 | 3.3 | 100 | 100 | |
| Kerang, VIC | maize | 0.02 | 0.018 | 0.026 | 2.8 | 100 | 100 | |
| Melbourne, VIC | maize | 0.02 | 0.019 | 0.029 | 2.1 | 93 | 98 | |
| Narrabri, NSW | light trap | 0.02 | 0.014 | 0.028 | 2.9 | 89 | 100 | |
| Narrabri, NSW | cotton | 0.02 | 0.018 | 0.024 | 4.9 | 100 | 100 | |
| Wee Waa, NSW | cotton | 0.02 | 0.018 | 0.029 | 3.2 | 96 | 100 | |
| Moree, NSW | cotton | 0.02 | 0.015 | 0.023 | 2.9 | 94 | 100 | |
| Wee Waa, NSW | cotton | 0.02 | 0.018 | 0.026 | 3.3 | 100 | 100 | |
| Narrabri, NSW | cotton | 0.02 | 0.020 | 0.028 | 2.7 | 89 | 98 | |
| Moree, NSW | sorghum | 0.02 | 0.018 | 0.032 | 2.5 | 95 | 98 | |
| Moree, NSW | sorghum | 0.02 | 0.013 | 0.021 | 2.5 | | 98 | |
| Emerald, Q | cotton | 0.02 | 0.019 | 0.029 | 3.1 | 97 | 100 | |
| Emerald, Q | cotton | 0.02 | 0.017 | 0.030 | 2.6 | | 96 | |
| Moree, NSW | cotton | 0.02 | 0.010 | 0.025 | 2.8 | 95 | 100 | |
| Moree, NSW | cotton | 0.03 | 0.022 | 0.033 | 3.4 | 100 | 100 | |
| Wee Waa, NSW | cotton | 0.03 | 0.026 | 0.035 | 3.1 | 95 | 100 | |
| Darwin, NT | pumpkin | 0.03 | 0.022 | 0.034 | 2.9 | 95 | 100 | |
| Emerald, Q | cotton | 0.03 | 0.024 | 0.035 | 4.0 | 100 | 100 | |
| Dalby, Q | cotton | 0.03 | 0.020 | 0.034 | 2.9 | 97 | 100 | |
| Dalby, Q | cotton | 0.03 | 0.025 | 0.045 | 2.4 | 86 | 100 | |
| Wee Waa, NSW | cotton | 0.03 | 0.022 | 0.041 | 3.2 | 91 | 100 | |
| Narrabri, NSW | cotton | 0.03 | 0.026 | 0.040 | 3.0 | 97 | 100 | |
| Laboratory Susceptible | Colony | 0.03 | 0.026 | 0.041 | 3.0 | 92 | 100 | |
| Wee Waa, NSW | sunflowers | 0.03 | 0.026 | 0.038 | 3.5 | 97 | 100 | |
| Gatton, Q | sorghum | 0.03 | 0.028 | 0.038 | 3.5 | 96 | | |
| Emerald, Q | maize | 0.04 | 0.029 | 0.043 | 2.4 | 78 | 96 | |
| Wee Waa, NSW | sunflowers | 0.04 | 0.033 | 0.048 | 2.9 | 88 | 95 | |
| Wee Waa, NSW | maize | 0.04 | 0.030 | 0.048 | 2.3 | 80 | 100 | |
| Wee Waa, NSW | maize | 0.04 | 0.034 | 0.048 | 3.0 | 89 | 100 | |
| Wee Waa, NSW | maize | 0.04 | 0.036 | 0.050 | 2.5 | 65 | 98 | |
| Mareeba, Q | tobacco | 0.04 | 0.033 | 0.051 | 3.7 | 86 | 100 | |
| Narrabri, NSW | light trap | 0.04 | 0.034 | 0.052 | 2.5 | 87 | | |
| Narrabri, NSW | light trap | 0.04 | 0.032 | 0.053 | 3.0 | 91 | | |
| Dalby, Q | cotton | 0.04 | 0.029 | 0.053 | 2.4 | 75 | 100 | |
| Emerald, Q | cotton | 0.04 | 0.027 | 0.046 | 2.8 | 85 | 97 | |
| Wee Waa, NSW | maize | 0.05 | 0.039 | 0.052 | 2.8 | 85 | 97 | |
| Moree, NSW | cotton | 0.05 | 0.044 | 0.067 | 2.9 | 83 | 97 | |
| **Average ± standard error** | | **0.030 ± 0.0015** | | | **3.0 ± 0.09** | **91.0 ± 1.4** | **99.1 ± 0.3** | |

### 40 - 60 MG LARVAE

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Narrabri, NSW | cotton | 0.03 | 0.026 | 0.038 | 3.5 | | 100 | 100 |
| Moree, NSW | sorghum | 0.04 | 0.034 | 0.055 | 2.9 | | 97 | 100 |
| Darwin, NT | pumpkin | 0.05 | 0.042 | 0.061 | 3.2 | | 100 | 100 |
| Narrabri, NSW | cotton | 0.06 | 0.045 | 0.073 | 2.2 | | 100 | 100 |
| Dalby, Q | cotton | 0.07 | 0.051 | 0.089 | 2.2 | | 80 | 100 |
| Laboratory Susceptible | Colony | 0.08 | 0.065 | 0.100 | 2.1 | | 80 | |
| Emerald, Q | cotton | | | | | | 87 | 98 |
| **Average ± standard error** | | **0.055 ± 0.0076** | | | **2.7 ± 0.24** | | **92.0 ± 3.6** | **99.7 ± 0.3** |

**Table A2.1** Calibration of fenvalerate bioassays and discriminating doses (for two larval weight ranges) on various strains of susceptible *Heliothis armigera* collected as eggs or larvae on a range of hosts from 1983 to 1984 and tested in the F1-3.

| Bioassay of Susceptible Larval *Heliothis armigera* : DELTAMETHRIN | | | | | |
|---|---|---|---|---|---|
| Strain Data | | LD50 | 95% Conf Interval | | |
| Collection Site | Host | (µg/30-40 mg larva) | Lower | Upper | Slope |
| Moree, NSW | cotton | 0.004 | 0.0020 | 0.0057 | 1.6 |
| Narrabri, NSW | cotton | 0.005 | 0.0034 | 0.0066 | 1.5 |
| Kerang, VIC | maize | 0.005 | 0.0038 | 0.0069 | 1.5 |
| Gatton, Q | sorghum | 0.005 | 0.0038 | 0.0061 | 1.8 |
| Darwin, NT | pumpkin | 0.005 | 0.0033 | 0.0066 | 1.5 |
| Wee Waa, NSW | cotton | 0.006 | 0.0050 | 0.0080 | 1.4 |
| Narrabri, NSW | cotton | 0.006 | 0.0047 | 0.0075 | 1.7 |
| Laboratory Susceptible Colony | | 0.006 | 0.0048 | 0.0080 | 2.0 |
| Moree, NSW | sorghum | 0.007 | 0.0042 | 0.0112 | 1.2 |
| Melbourne, VIC | maize | 0.008 | 0.0064 | 0.0108 | 1.7 |
| Wee Waa, NSW | cotton | 0.009 | 0.0057 | 0.0177 | 0.9 |
| Emerald, Q | cotton | 0.009 | 0.0061 | 0.0124 | 1.5 |
| Narrabri, NSW | cotton | 0.009 | 0.0066 | 0.0177 | 1.7 |
| Moree, NSW | sunflowers | 0.011 | 0.0086 | 0.0145 | 1.3 |
| Narrabri, NSW | cotton | 0.011 | 0.0089 | 0.0144 | 2.3 |
| Moree, NSW | sorghum | 0.012 | 0.0088 | 0.0157 | 2.1 |
| Moree, NSW | sunflowers | 0.012 | 0.0089 | 0.0157 | 1.8 |
| Wee Waa, NSW | cotton | 0.013 | 0.0098 | 0.0173 | 2.4 |
| Wee Waa, NSW | cotton | 0.013 | 0.0096 | 0.0180 | 2.0 |
| Mareeba, Q | tobacco | 0.014 | 0.0080 | 0.0199 | 1.7 |
| Narrabri, NSW | cotton | 0.016 | 0.0120 | 0.0200 | 2.1 |
| **Average ± standard error** | | **0.009 ± 0.0008** | | | **1.7 ± 0.08** |

**Table A2.2** Calibration of deltamethrin bioassays on various strains of susceptible *Heliothis armigera* collected as eggs or larvae on a range of hosts from 1983 to 1984 and tested in the F1-3.

## Bioassay of Susceptible Larval *Heliothis armigera* : CYCLODIENES

### ENDOSULFAN

| Strain Data | | LD50 | 95% Conf Interval | | | % Kill @ µg/larva | |
|---|---|---|---|---|---|---|---|
| Collection Site | Host | (µg/30-40 mg larva) | Lower | Upper | Slope | 5.0 | 10.0 |
| Laboratory Susceptible Colony | | 0.49 | 0.33 | 0.65 | 2.4 | 100 | 100 |
| Narrabri, NSW | cotton | 0.59 | 0.48 | 0.73 | 3.0 | 100 | 100 |
| Wee Waa, NSW | cotton | 0.68 | 0.52 | 0.84 | 2.7 | 100 | 100 |
| Laboratory Susceptible Colony | | 0.70 | 0.56 | 0.92 | 2.2 | 98 | 100 |
| Dalby, Q | cotton | 0.73 | 0.27 | 1.29 | 1.0 | 79 | 90 |
| Narrabri, NSW | cotton | 0.79 | 0.35 | 1.19 | 1.8 | 90 | 95 |
| Moree, NSW | sorghum | 0.84 | 0.76 | 1.16 | 3.6 | 95 | 100 |
| Darwin, NT | pumpkin | 0.96 | 0.77 | 1.20 | 3.1 | 100 | 100 |
| Moree, NSW | sunflowers | 0.98 | 0.81 | 1.19 | 3.7 | 98 | 100 |
| **Average** | | **0.75** | | | **2.6** | **95.6** | **98.3** |
| **± standard error** | | **± 0.054** | | | **± 0.29** | **± 2.4** | **± 1.2** |

### DIELDRIN

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Laboratory Susceptible Colony | | 2.86 | 2.17 | 3.81 | 1.8 | | |

### ENDRIN

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Laboratory Susceptible Colony | | 0.60 | 0.456 | 0.761 | 2.0 | | |

**Table A2.3** Calibration of cyclodiene (endosulfan, dieldrin & endrin) bioassays and endosulfan discriminating doses on various strains of susceptible *Heliothis armigera* collected as eggs or larvae on a range of hosts from 1983 to 1984 and tested in the F1-3.

**Repeated Bioassay on a Susceptible Laboratory Colony of *Heliothis armigera***

| Chemical | Generation Tested | LD50 (µg/30-40 mg larva) | 95% Conf Interval Lower | Upper | Slope |
|---|---|---|---|---|---|
| Fenvalerate | 34 | 0.03 | 0.028 | 0.038 | 3.5 |
| | 42 | 0.03 | 0.026 | 0.041 | 3.0 |
| | 58 | 0.03 | 0.024 | 0.034 | 3.2 |
| | 59 | 0.03 | 0.026 | 0.037 | 3.4 |
| | 60 | 0.04 | 0.036 | 0.048 | 3.3 |
| | 61 | 0.02 | 0.017 | 0.025 | 3.5 |
| | 75 | 0.03 | 0.025 | 0.039 | 2.4 |
| | 76 | 0.03 | 0.025 | 0.033 | 3.1 |
| | 79 | 0.03 | 0.028 | 0.040 | 3.0 |
| | 83 | 0.03 | 0.021 | 0.029 | 3.4 |
| | 88 | 0.04 | 0.035 | 0.046 | 3.5 |
| | 94 | 0.03 | 0.023 | 0.031 | 3.6 |
| Deltamethrin | 34 | 0.006 | 0.0047 | 0.0075 | 1.7 |
| | 35 | 0.006 | 0.0048 | 0.0080 | 2.0 |
| | 43 | 0.005 | 0.0038 | 0.0061 | 1.8 |
| | 59 | 0.004 | 0.0033 | 0.0053 | 2.1 |
| | 60 | 0.004 | 0.0032 | 0.0046 | 2.9 |
| | 81 | 0.013 | 0.0110 | 0.0149 | 2.3 |
| | 94 | 0.009 | 0.0075 | 0.0108 | 2.3 |
| Endosulfan | 34 | 0.70 | 0.56 | 0.92 | 2.2 |
| | 43 | 0.49 | 0.33 | 0.65 | 2.4 |
| | 59 | 0.46 | 0.39 | 0.54 | 3.8 |
| | 76 | 0.50 | 0.41 | 0.61 | 2.7 |
| | 95 | 0.69 | 0.60 | 0.79 | 3.7 |

**Table A2.4** Consistency of bioassay results on a long term laboratory culture of pyrethroid and endosulfan susceptible *Heliothis armigera*, collected originally off sorghum at Gatton, Qld in 1979 and reared subsequently on artificial diet without any further infusion of field material. (Colony maintained by Dr.R.Teakle, Department of Primary Industries, Brisbane, Qld.). Colony bioassayed at irregular intervals after the 34th generation (approx 8 generations per year in lab).

**Bioassay of Susceptible Larval *Heliothis armigera* : RESISTANCE BREAKING PYRETHROID**

| Date Tested | $LD_{50}$ (µg/30-40 mg larva) | 95% Conf Interval Lower | 95% Conf Interval Upper | Slope | % Kill @ µg/larva 0.07 | 0.1 | 0.2 |
|---|---|---|---|---|---|---|---|
| Aug 1989 | 0.015 | 0.0129 | 0.0174 | 3.4 | | | |
| Jan 1990 | 0.031 | 0.0277 | 0.0349 | 2.8 | | | |
| Feb 1990 | 0.029 | 0.0259 | 0.0312 | 2.7 | 92.6 n =759 | 96.7 n =1,212 | 99.9 n =1,000 |
| May 1990 | 0.019 | 0.0170 | 0.0215 | 3.3 | | | |
| **Average** | **0.024** | | | **3.1** | | | |
| **± standard error** | **± 0.0039** | | | **± 0.18** | | | |

**Table A2.5** Calibration of Resistance Breaking Pyrethroid bioassays and discriminating doses on 4 generations of the laboratory susceptible *Heliothis armigera* colony. n = total number of larvae tested at each candidate discriminating dose.

## Bioassay of Susceptible Larval *Heliothis punctigera*

### FENVALERATE

| Strain Data | | LD50 | 95% Conf Interval | | | % Kill @ µg/larva | |
|---|---|---|---|---|---|---|---|
| Collection Site | Host | (µg/30-40 mg larva) | Lower | Upper | Slope | 0.025 | 0.05 |
| Narrabri, NSW | cotton | 0.008 | 0.0064 | 0.0088 | 4.3 | 97.4 | 100 |
| Narrabri, NSW | cotton | 0.009 | 0.0076 | 0.0117 | 3.3 | 92.0 | 100 |
| Emerald, Q | cotton | 0.009 | 0.0076 | 0.0106 | 3.5 | 92.5 | 100 |
| Moree, NSW | cotton | 0.010 | 0.0083 | 0.0114 | 4.3 | 94.9 | 100 |
| Emerald, Q | cotton | 0.011 | 0.0091 | 0.0124 | 3.2 | 86.3 | 98.3 |
| Mareeba, Q | tobacco | 0.011 | 0.0078 | 0.0138 | 3.2 | 85.0 | 100 |
| Bourke, NSW | cotton | 0.013 | 0.0108 | 0.0156 | 2.7 | 74.0 | 97.6 |
| Narrabri, NSW | geraniums | 0.014 | 0.0120 | 0.0164 | 4.3 | 88.2 | 97.1 |
| Long Term Laboratory Colony | | 0.014 | 0.0121 | 0.0170 | 3.0 | 76.0 | 98.0 |
| Emerald, Q | cotton | 0.015 | 0.0126 | 0.0177 | 3.7 | 72.7 | 100 |
| **Average ± standard error** | | **0.011 ± 0.0008** | | | **3.6 ± 0.18** | **85.9 ± 2.8** | **99.1 ± 0.4** |

### ENDOSULFAN

| Strain Data | | LD50 | 95% Conf Interval | | | % Kill @ |
|---|---|---|---|---|---|---|
| Collection Site | Host | (µg/30-40 mg larva) | Lower | Upper | Slope | 2.5 µg/larva |
| Narrabri, NSW | cotton | 0.37 | 0.310 | 0.430 | 3.9 | 100 |
| Narrabri, NSW | cotton | 0.42 | 0.356 | 0.496 | 3.4 | 100 |
| Long Term Laboratory Colony | | 0.47 | 0.398 | 0.553 | 3.5 | 100 |
| Emerald, Q | cotton | 0.53 | 0.458 | 0.619 | 3.4 | 97.9 |
| Moree, NSW | sunflowers | 0.68 | 0.542 | 0.839 | 3.3 | 96.2 |
| Moree, NSW | cotton | 0.73 | 0.642 | 0.840 | 4.0 | 100 |
| Emerald, Q | cotton | 0.81 | 0.708 | 0.927 | 3.5 | 97.9 |
| Bourke, NSW | cotton | 0.82 | 0.689 | 1.009 | 3.5 | 90 |
| Moree, NSW | sunflowers | 0.83 | 0.684 | 1.014 | 2.8 | 90 |
| Mareeba, Q | Tobacco | 0.92 | 0.706 | 1.230 | 3.1 | 88.9 |
| Narrabri, NSW | geraniums | 0.97 | 0.822 | 1.149 | 3.7 | 94.7 |
| **Average ± standard error** | | **0.69 ± 0.063** | | | **3.5 ± 0.10** | **96.0 ± 1.3** |

**Table A2.6** Calibration of fenvalerate and endosulfan bioassays and discriminating doses on various strains of susceptible *Heliothis punctigera* collected as eggs or larvae on a range of hosts from 1983-85 and tested in the F1-2.

## Bioassay of Susceptible Adult *Heliothis armigera* : FENVALERATE

### TOPICAL EYE TEST

| Date tested | Sex | Av. weight of 1 day old, fed, unmated moths (mg) ± s.e. | LD50 (µg/moth) | 95% Conf Interval Lower | 95% Conf Interval Upper | Slope | % Kill @ µg/moth 1.0 | % Kill @ µg/moth 2.0 |
|---|---|---|---|---|---|---|---|---|
| Nov 1985 | ♀ | 211 ± 2.8 | 0.29 | 0.22 | 0.36 | 2.5 | | |
| | ♂ | 193 ± 2.1 | 0.35 | 0.25 | 0.49 | 1.5 | 82.8 n =99 | 100 n =91 |
| Nov 1986 | ♀ | 247 ± 2.4 | 0.31 | 0.24 | 0.43 | 2.3 | | |
| | ♂ | 218 ± 2.2 | 0.45 | 0.34 | 0.63 | 2.6 | | |
| **Average ± standard error** | ♀ | | **0.30 ± 0.010** | | | **2.4 ± 0.10** | | |
| | ♂ | | **0.40 ± 0.050** | | | **2.1 ± 0.55** | | |

### TARSAL PLATE TEST

| Date tested | Sex | Av. weight of 1 day old, fed, unmated moths (mg) ± s.e. | LC50 (µg/cm²) | 95% Conf Interval Lower | 95% Conf Interval Upper | Slope | % Kill @ µg/cm² 0.79 | % Kill @ µg/cm² 1.57 |
|---|---|---|---|---|---|---|---|---|
| Nov 1985 | ♀ | 240 ± 2.8 | 0.21 | 0.17 | 0.25 | 2.8 | | |
| | ♂ | 216 ± 2.1 | 0.28 | 0.23 | 0.32 | 3.0 | | |
| Nov 1986 | ♀ | 247 ± 2.4 | 0.27 | 0.17 | 0.45 | 2.1 | 93.5 n =170 | 98.3 n =120 |
| | ♂ | 218 ± 2.2 | 0.25 | 0.20 | 0.31 | 3.2 | | |
| Nov 1988 | ♀ | 244 ± 2.3 | 0.14 | 0.11 | 0.18 | 2.5 | | |
| | ♂ | 231 ± 2.8 | 0.24 | 0.19 | 0.31 | 2.3 | | |
| **Average ± standard error** | ♀ | | **0.21 ± 0.038** | | | **2.5 ± 0.20** | | |
| | ♂ | | **0.26 ± 0.012** | | | **2.8 ± 0.27** | | |

**Table A2.7** Calibration of fenvalerate bioassays and discriminating doses (for two testing techniques) on adults of the laboratory susceptible *Heliothis armigera* colony, tested between 1985 and 1988. Moths dosed either directly on the eye (topical eye test) or indirectly, by enforced contact with treated glass plates (tarsal plate test). LD & LC50's adjusted to standard 200 mg moth. n = total number of moths tested at each candidate discriminating dose.

# APPENDIX 3 - Insecticide Use Surveys

One of the most important factors affecting resistance frequency is selection pressure. Thus it is important to determine the intensity and pattern of insecticide use within each study area. Such a survey also serves as an indicator of the compliance rate with the voluntary Strategy.

## Methods

Each season, growers or consultants were surveyed as to the insecticide use on their properties falling within the Namoi/Gwydir and Emerald study areas. Each grower or consultant was asked to document the area sprayed, the insecticide/s and rates used and the total area of cotton sown. The survey was not just confined to sprays put on for *Heliothis* spp. but also included sprays put on for other pests (such as tipworm, rough bollworm and mites) which could also impact on any *Heliothis* present. However, it did not include organophosphate sprays for thrips, mirids or aphids (eg. dimethoate, omethoate) which are ineffective against *Heliothis* spp. Most properties (90-100%) in each study area participated in the surveys. Selection pressure was expressed as the number of sprays per hectare (total hectares sprayed ÷ total cotton area), not as sprays per farm, as this would have biased the figures towards the smaller properties. Also, early season ground rig band applications were included on a sown area basis and not just as the area of the treated band.

## Results & Discussion

### *Total insecticide use* (Fig.A3.1)

The total number of *Heliothis* sprays per season varied from 7-11, depending on the infestation level. Generally higher pest pressure in the northern Emerald Irrigation Area required one half to two sprays more than the Namoi/Gwydir.

### *Insecticide Use by Stage* (Fig. A3.2)

Most insecticide use was in Stage 1 (47% in the Namoi/Gwydir averaged over the last 6 seasons and 43% in the Emerald Irrigation Area averaged over the last 3 seasons). The 1985/86 season at Emerald was excluded from this analysis as the Stage 2 pyrethroid window for that season was positioned one month later than in subsequent years. This was done intentionally to allow the gradual phasing in of pyrethroids after their withdrawal following the widespread field resistance problems in the 1982/83 Emerald season.

Insecticide use in Stage 2 was slightly lower than in Stage 1 (40 & 35% overall average in the Namoi/Gwydir and Emerald, respectively). Because of the high cost of Stage 3 insecticides (Table A3.1), there is a strong incentive for growers to avoid using Stage 3 insecticides. Consequently, Stage 3 insecticide use was the lowest (13 & 22% overall average in the Namoi/Gwydir and Emerald, respectively).

### *Endosulfan - Namoi/Gwydir* (Figs. A3.3 & 4)

Three to four endosulfan sprays were used in Stage 1, with a smaller amount (about half a spray) being used in the Stage 2 pyrethroid window. Consistent with the Strategy recommendations, no

endosulfan was used on cotton in Stage 3. Growers and consultants were at first very tentative in accepting endosulfan as an effective insecticide against *Heliothis* (only 75% and 81% use in Stage 1 in the first two years of the Strategy). However, endosulfan proved to be a reliable, cost effective insecticide for *Heliothis* control and the industry accepted it so well, that it is now virtually the sole insecticide used against *Heliothis* in Stage 1. It also retains a small but consistent use in Stage 2 at about 15% of Stage 2 sprays. It is used mainly to break up the pyrethroid sprays and was the main product chosen to replace pyrethroids when they performed poorly due to resistance problems in the 1986/87 late Stage 2 period. The Stage 2 data also indicate that growers and consultants were tentative in accepting endosulfan in the first year of the Strategy preferring organophosphates to relieve their anxiety about the performance of the pyrethroids. However, as their confidence in endosulfan grew, the use of the expensive organophosphates declined and endosulfan came to be regarded as the preferred break chemical in Stage 2. In fact, endosulfan would now account for approximately half of all the sprays used against *Heliothis* in cotton.

### *Endosulfan - Emerald* (Figs. A3.5 & 6)

As in the Namoi/Gwydir, endosulfan is by far the most commonly used insecticide in cotton. Stage 1 use was similar to the Namoi/Gwydir but somewhat more variable (2 to 5 sprays). The higher use in 1985/86 season was no doubt due to the later timing of the Stage 2 pyrethroid window in that season. Data for Stage 2 clearly indicate an increasing reliance on endosulfan concomitant with decreasing use of pyrethroids and organophosphates. This is probably due to the reduced reliability of the pyrethroids under the higher *Heliothis armigera* pressure at Emerald alongwith the greater cost effectiveness of endosulfan over the organophosphates. The increase in confidence in the efficacy of endosulfan noted in the Namoi/Gwydir, was also clearly evident at Emerald, both in Stages 1 and 2. There was also no endosulfan use in the Stage 3 closed period, indicating excellent compliance with the voluntary Strategy.

### *Pyrethroids - Namoi/Gwydir* (Figs. A3.3, 4 & 7)

Two to three pyrethroid sprays were applied in Stage 2. As recommended in the voluntary Strategy, no pyrethroids were applied outside of the pyrethroid window. In the first year of the Strategy (1983/84 season) growers and consultants were somewhat reluctant to use the pyrethroids (63% of Stage 2 sprays). However, by the next season, their new found confidence in the Strategy's ability to contain pyrethroid resistance, saw pyrethroid use rise quickly to 81% of Stage 2 sprays and except for the 1986/87 season, pyrethroid use has stabilised at around this level. The lower acceptance of pyrethroids in the high pressure 1986/87 season was due to the occurrence of several pyrethroid resistance spray failures in the late Stage 2 period, with a consequent swing to alternatives, principally endosulfan.

For the first three seasons of the Strategy, deltamethrin and cypermethrin were the most commonly used pyrethroids. However, after the introduction of lambdacyhalothrin in the 1986/87 season, cypermethrin use plummeted and it has now been replaced by its resolved isomer alphacypermethrin. Deltamethrin use also continued to decline but at a much slower rate. Fenvalerate use has increased slightly (10-20%) and it has just been replaced by its resolved isomer esfenvalerate.

Lambdacyhalothrin use has gradually increased since its introduction and it is now the most popular pyrethroid used on Namoi/Gwydir cotton, accounting for just over half of all pyrethroids used.

### *Pyrethroids - Emerald* (Figs. A3.5, 6 & 7)

After the 1982/83 field failures at Emerald, pyrethroids were withdrawn for one season (1983/84) and then phased in gradually over the following two seasons (1984/85 & 1985/86) by intentionally positioning the pyrethroid window towards the end of the season. Consequently, pyrethroid use at Emerald was nil to low (maximum half a spray in 1985/86 season) for the three seasons following the 1982/83 field failures. However, after the repositioning of the Stage 2 pyrethroid window to the preferred opening date of January 1st at Emerald, pyrethroid use quickly resurged to levels similar to the Namoi/Gwydir, mainly to relieve the intense selection pressure on endosulfan which had occurred over the previous three seasons. However, because of the greater *Heliothis armigera* pressure at Emerald, pyrethroid use has decreased slightly since and has stabilised at a lower level than the Namoi/Gwydir (about 50% of Stage 2 sprays).

Patterns in pyrethroid selection were similar to the Namoi/Gwydir except that the market seemed much more volatile and responsive to local supply and pricing policies etc. No single pyrethroid clearly dominated the market for more than one season.

### *Organophosphates/Carbamates - Namoi/Gwydir & Emerald* (Figs. A3.3, 4,5 & 6)

Organophosphates and carbamates were used in all three stages but their main use period was in Stage 3 when both endosulfan and pyrethroids are not recommended. In this Stage, organophosphates predominated in the Namoi/Gwydir (90-100% of sprays) but greater reliance on carbamates (principally thiodicarb) at Emerald reduced this to 60-80% of Stage 3 sprays there.

Stage 3 insecticide use is normally quite variable due to unpredictable late season pest pressure. This varied from 0.5 to 2.0 sprays in the Namoi/Gwydir and 1.0 to 2.5 at Emerald. Because of the high cost of organophosphates and carbamates relative to pyrethroids and endosulfan, growers attempt to minimise use of these Stage 3 insecticides, wherever possible. The most commonly used organophosphate was profenofos (55% & 45% of organophosphate sprays in the Namoi/Gwydir and Emerald, respectively) followed by parathion (15% & 34%, also respectively). Minor use organophosphates were sulprofos (12% & 12%), monocrotophos (15% & 4%) and chlorpyrifos (3% & 5%), all respectively as above. The main carbamate used was thiodicarb (over two thirds of all carbamate sprays in both the Namoi/Gwydir and Emerald), with the balance being ovicidal rates of methomyl.

### *Use of mixtures - Namoi/Gwydir* (Fig. A3.8)

The use of ovicide/larvicide mixtures has been strongly advocated as a component of the Australian Resistance Management Strategy (see Chapter 1). When the Strategy was first introduced, the use of mixtures with both endosulfan and pyrethroids was quite high but this has declined with time. This was particularly apparent with endosulfan where use of mixtures dropped steadily from 74% in 1983/84 to 3% in 1988/89. A similar but less pronounced trend occurred with the pyrethroids. The reverse trend to increasing use of mixtures with pyrethroids in 1986/87 was due to the pyrethroid

resistance problems which occurred in the latter half of the State 2 window in that season. The main mixtures used were larvicide/ovicide mixtures (with methomyl or chlordimeform) or larvicide/miticide mixtures (with chlorpyrifos, monocrotophos or profenofos). Some mixtures of larvicides were also used (eg. endosulfan/parathion). The decline in reliance on mixtures over time was probably due to a combination of the following factors:

- increasing confidence in the ability of the Strategy to contain pyrethroid resistance.
- increasing confidence in the efficacy of endosulfan used alone.
- the ever present need to reduce costs.
- lower insect pressure in the last two seasons.
- increased awareness of the capacity of organophosphate/endosulfan mixtures to flare mites.
- withdrawal of the very effective ovicide chlordimeform after the 1985/86 season.

### *Use of mixtures - Emerald* (Fig. A3.8)

The trend to declining reliance on mixtures was also evident for endosulfan at Emerald. However, the trend for pyrethroids was completely the reverse and most likely reflects the greater *Heliothis armigera* pressure and hence resistance problems, at Emerald.

### *Comparison with Pre-Strategy Use Patterns*

Little data is available on insecticide use in the formerly unregulated system prior to the introduction of the Resistance Management Strategy. However, a number of publications on pest management in Emerald before 1983/84 indicate the dramatic change that has occurred since the Strategy's implementation. For the five seasons prior to the introduction of the Strategy in 1983/84, pyrethroids at Emerald accounted for 60-90% of the total sprays applied (currently about 20%), ranging from 5-10 sprays/season (currently 1.5-2.5), spread more or less equally over the entire 160 day growing period (currently a 35 day window) (Waite 1983, Waite & Murray 1981, Adams & Pyke 1983). Riley (1990) documented a very similar pre-Strategy use pattern for pyrethroids in USA cotton (70% of the total treatments applied for *Heliothis* and up to 8.4 sprays per season). Although no hard confirmatory data are available, it is generally agreed that most of the pre-Strategy Australian cotton industry had a broadly similar pattern to Emerald. Current pyrethroid use patterns in the Namoi/Gwydir are also similar to Emerald but at a slightly higher level ( 30-35% of total *Heliothis* sprays and up to 2-3 sprays per season ), probably because of the lower *Heliothis armigera* pressure in the south. Thus, it is quite clear that the implementation of the Australian Resistance Management Strategy has had a profound and lasting impact on insecticide use in Australian cotton.

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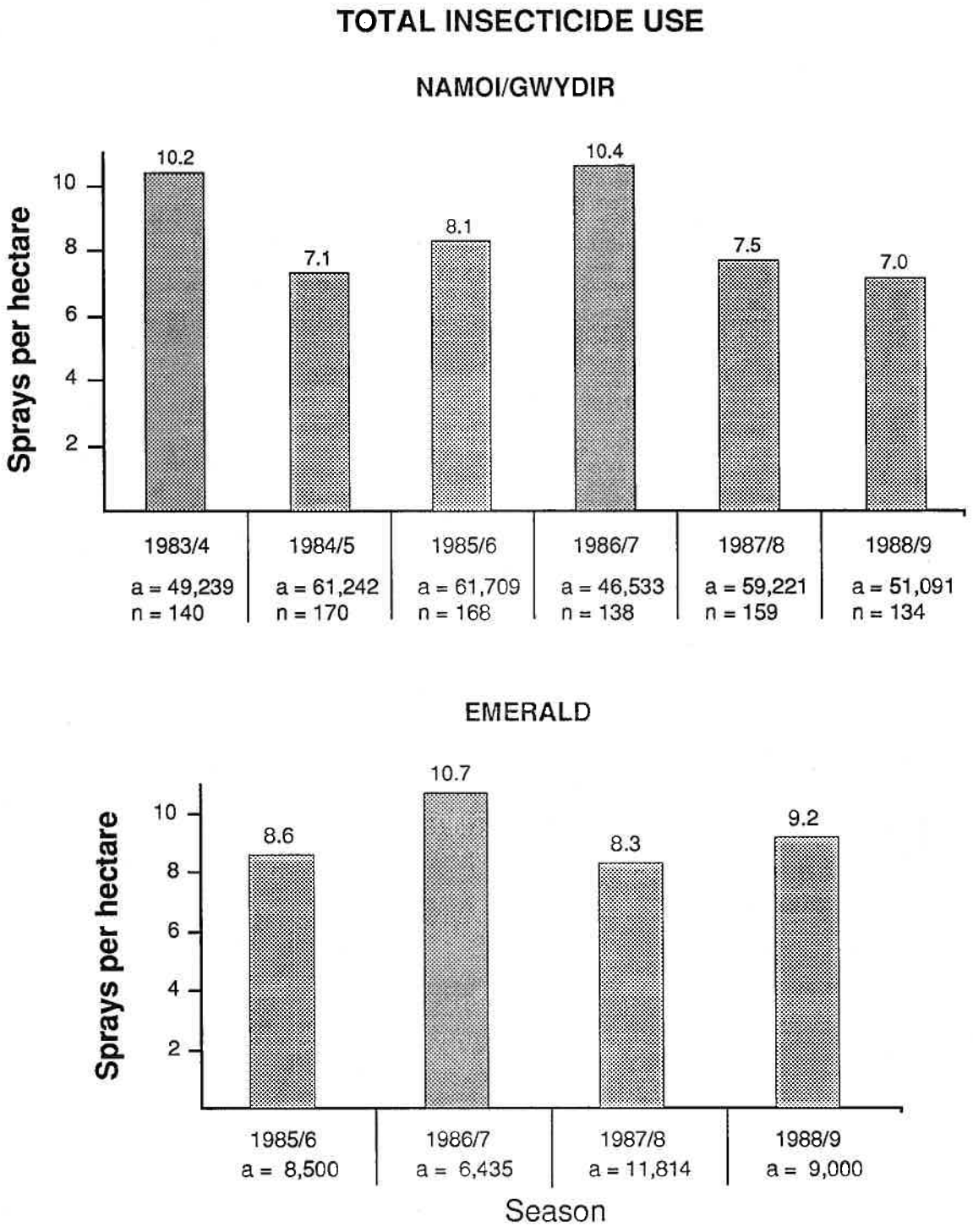


**Fig. A3.1** Total season useage (Stages I, II & III combined) of insecticides against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales and the Emerald Irrigation Area of central Queensland. a & n = the total number of hectares and properties surveyed, respectively.

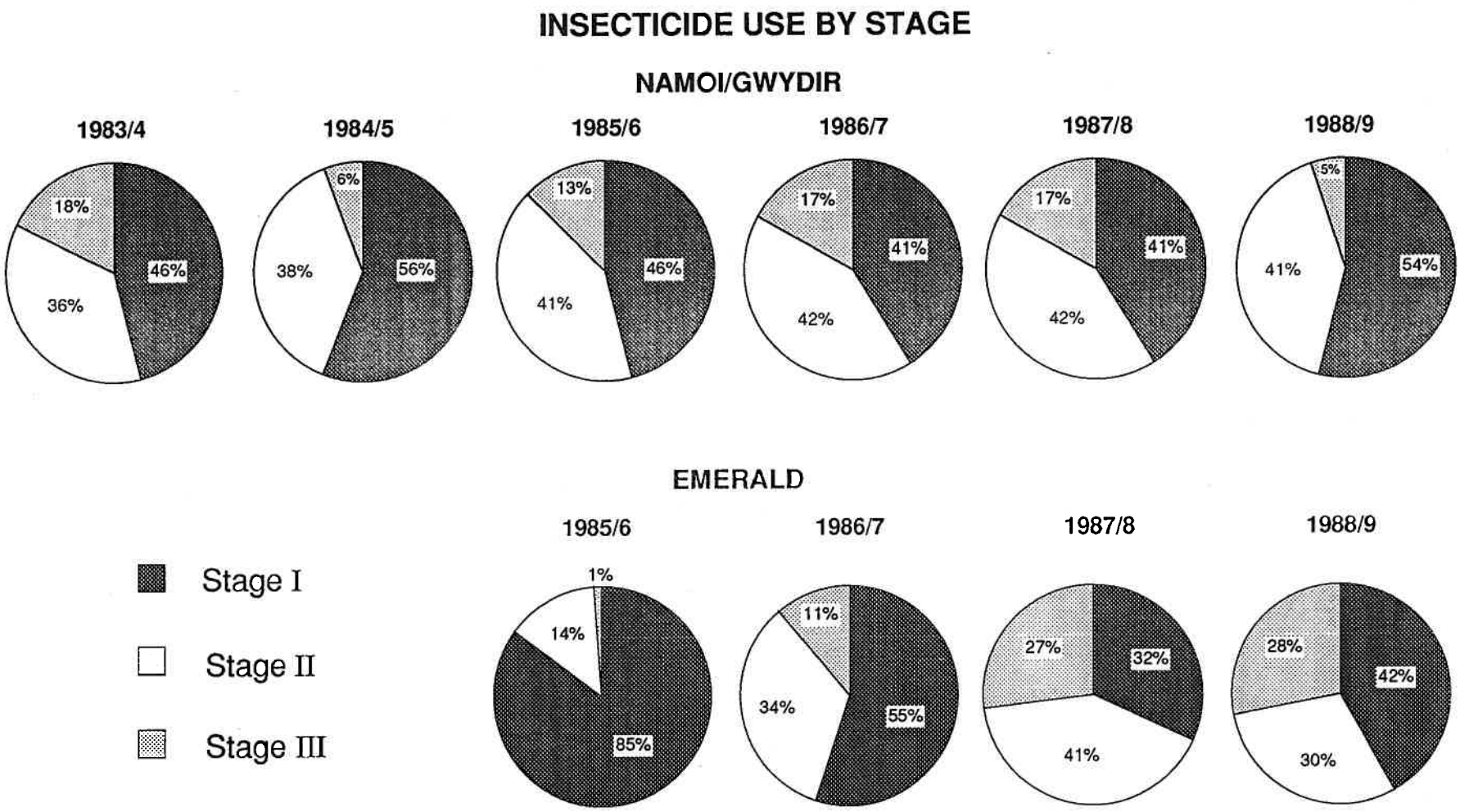


**Fig. A3.2** Breakdown of insecticide use against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales and the Emerald Irrigation Area of central Queensland, for each of the Stages (I, II & III) of the Resistance Management Strategy. N.B. 1985/86 Stage II period at Emerald was Feb 1 - Mar 14, all subsequent years years Jan 1 - Feb 14.

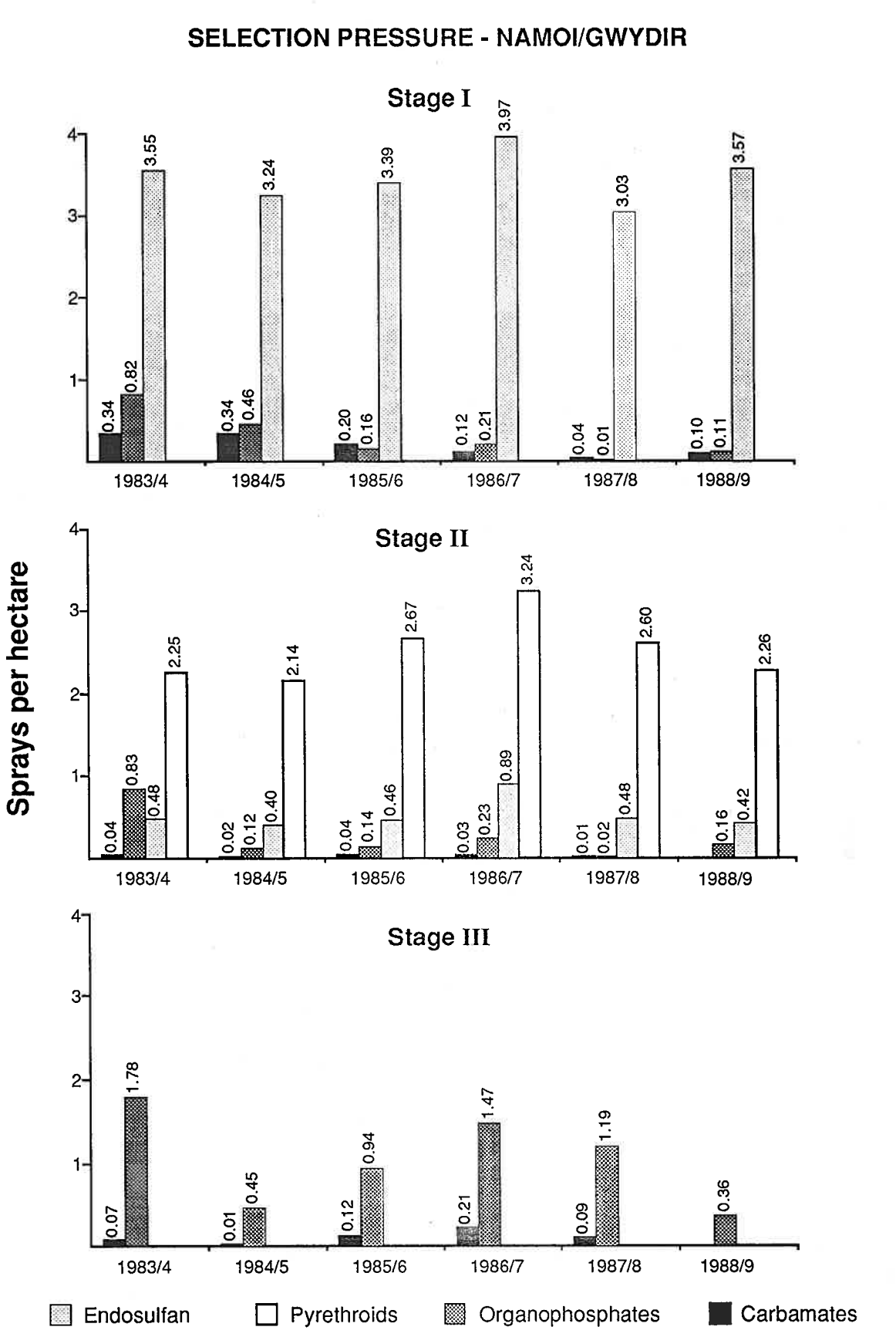


**Fig. A3.3** Spray intensity of various insecticide groups used within each Stage (I, II & III) against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales for the six seasons since the introduction of the Resistance Management Strategy.

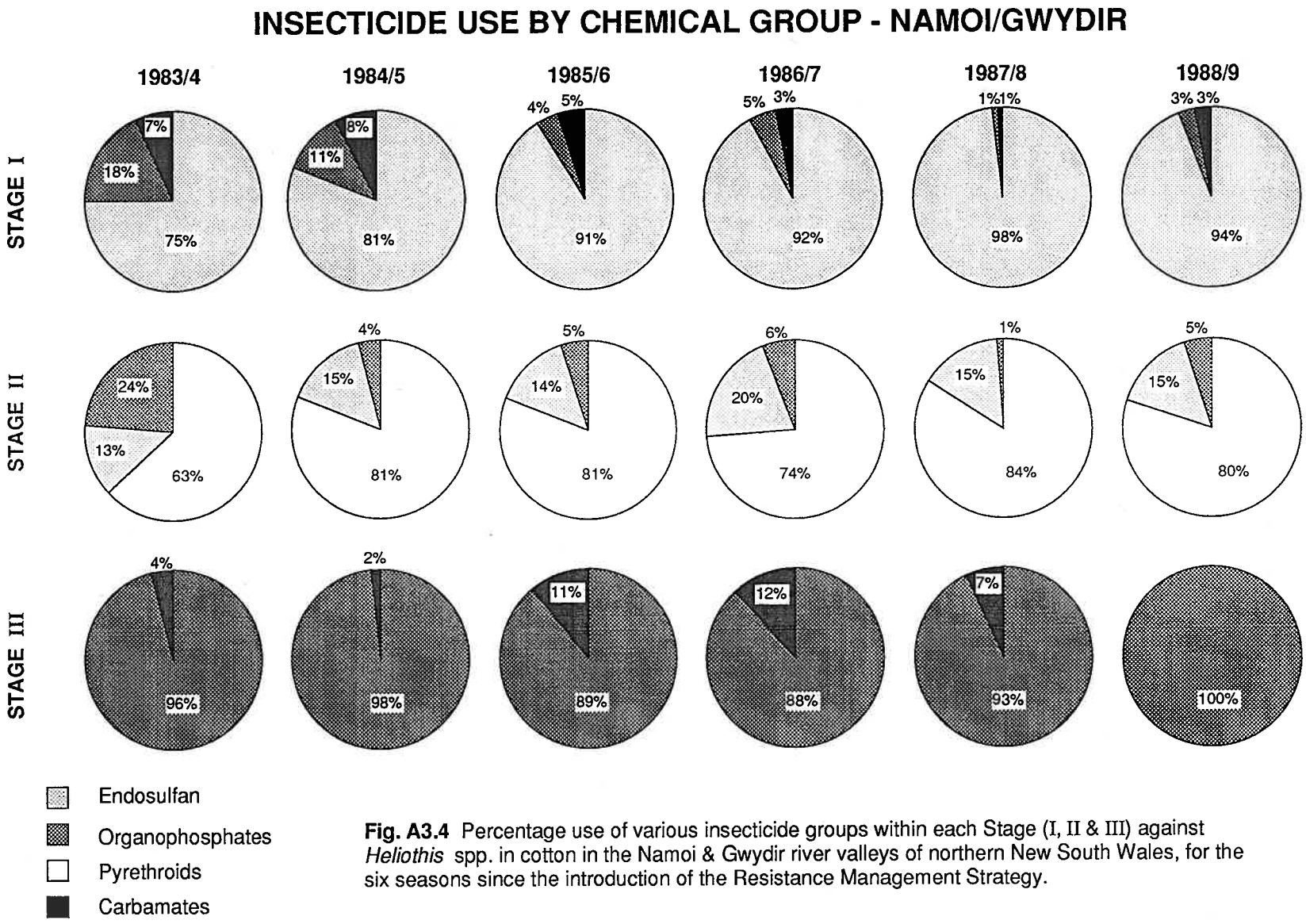


**Fig. A3.4** Percentage use of various insecticide groups within each Stage (I, II & III) against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales, for the six seasons since the introduction of the Resistance Management Strategy.

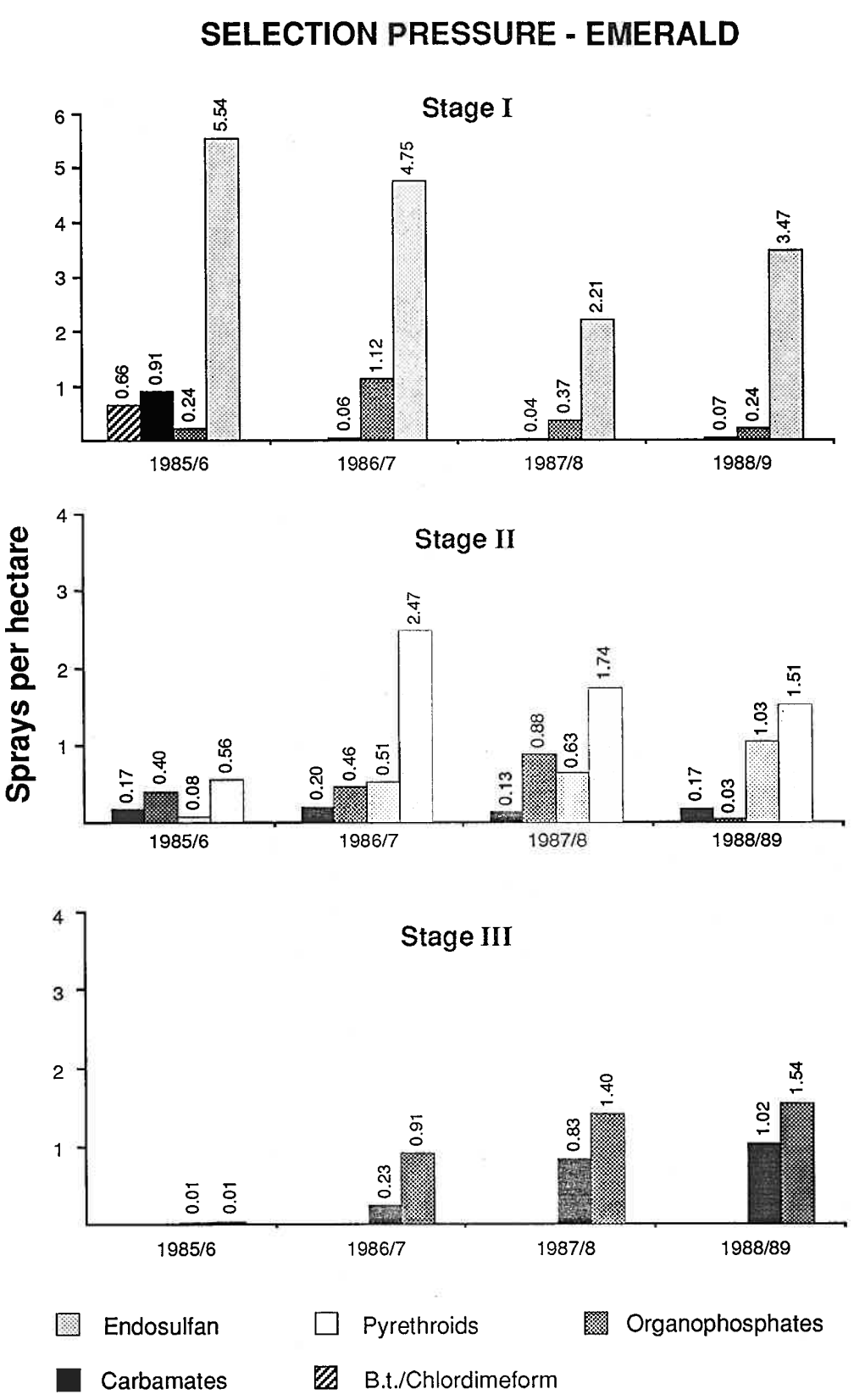


**Fig. A3.5** Spray intensity of various insecticide groups used within each Stage (I, II & III) against *Heliothis* spp. in cotton in the Emerald Irrigation Area of central Queensland for the past four seasons of the Resistance Management Strategy. N.B. 1985/86 Stage II period was Feb 1 - Mar 14, all subsequent years Jan 1 - Feb 10.

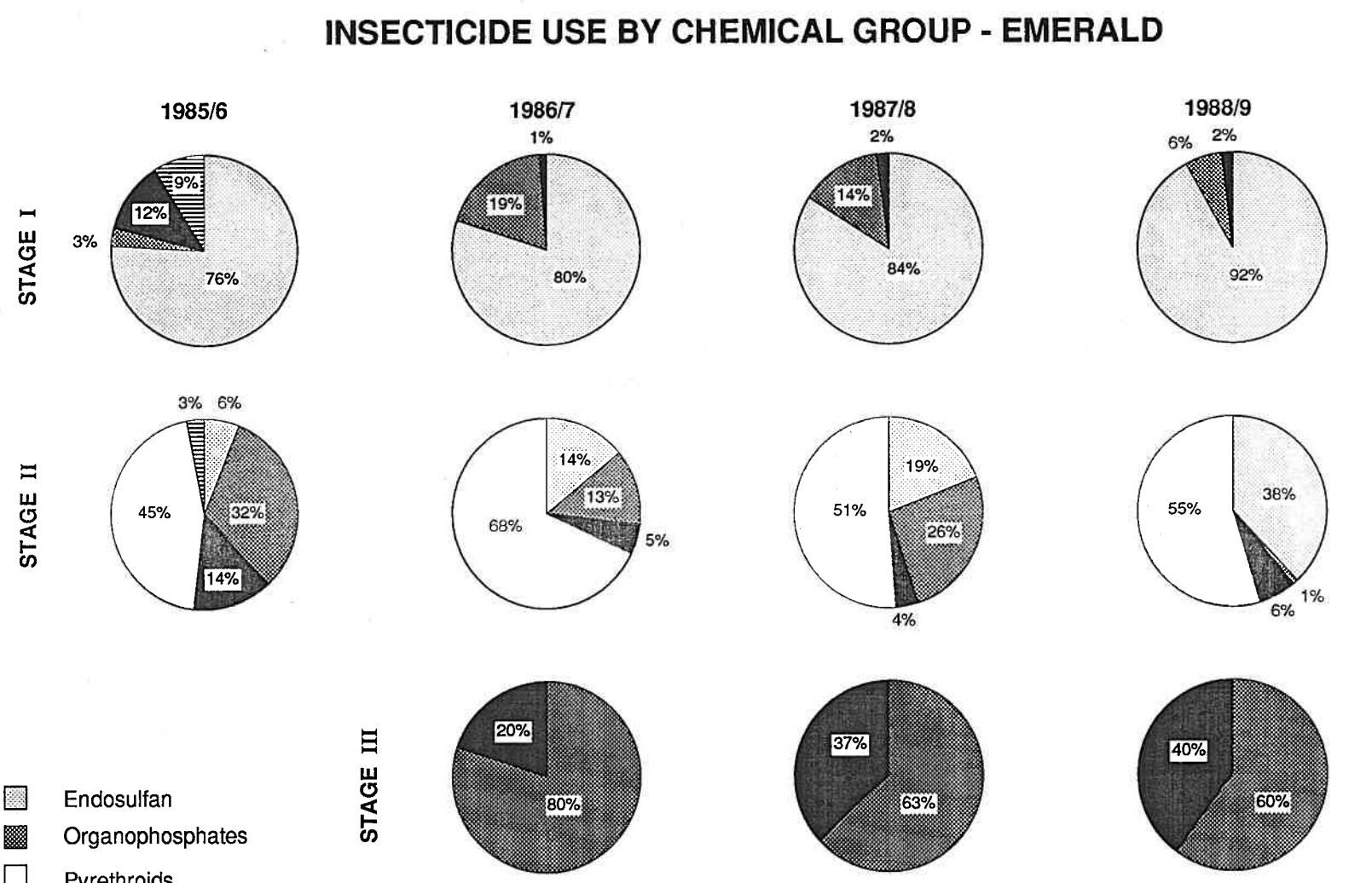


**Fig. A3.6** Percentage use of various insecticide groups within each Stage (I, II & III) against *Heliothis* spp. in cotton in the Emerald Irrigation Area of central Queensland, for the past four seasons of the Resistance Management Strategy. N.B.1985/86 Stage II period was Feb 1 - Mar 14, all subsequent years Jan 1 - Feb 10.

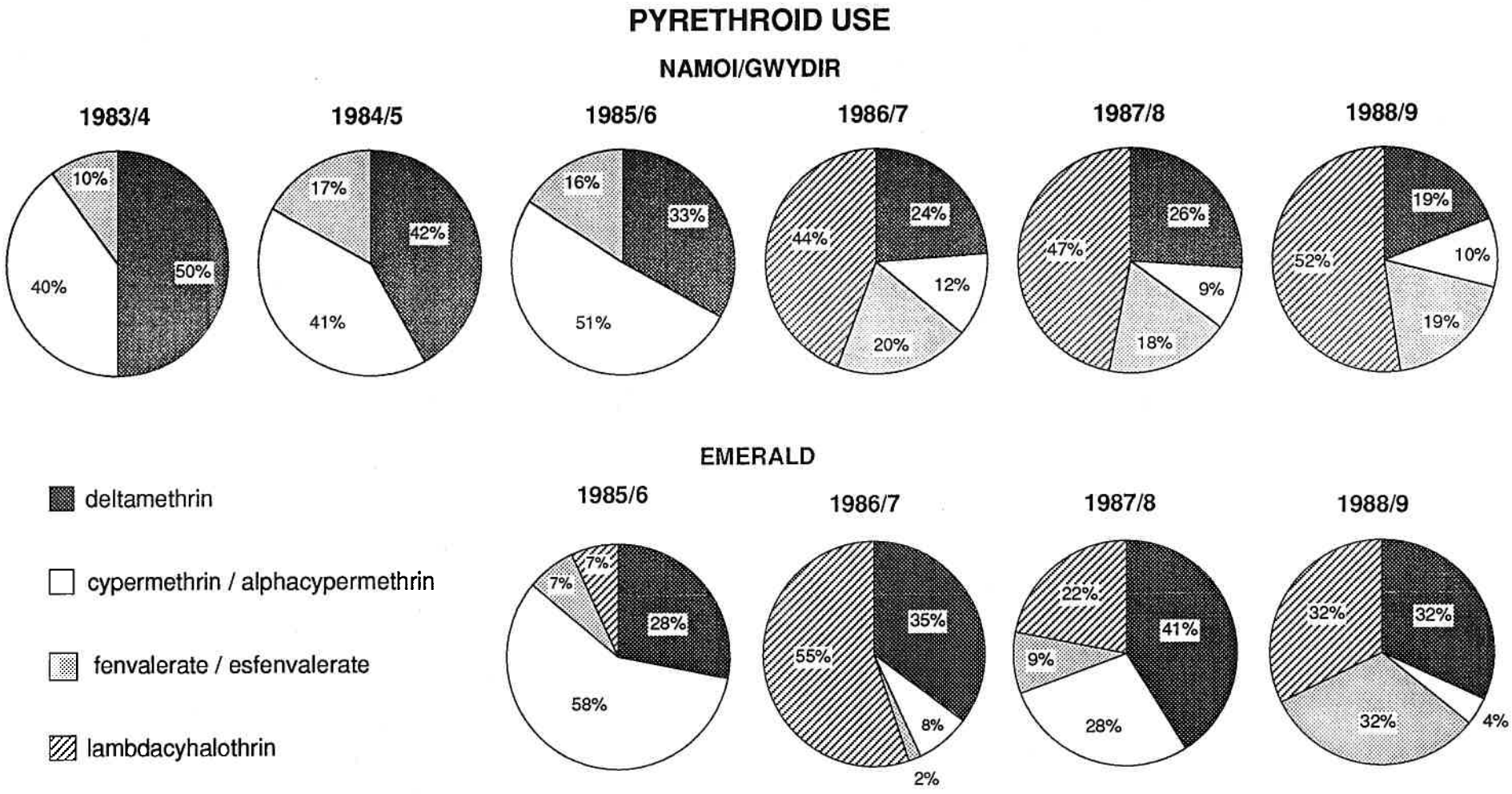


**Fig. A3.7** Breakdown of pyrethroid use against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales and the Emerald Irrigation Area of central Queensland.

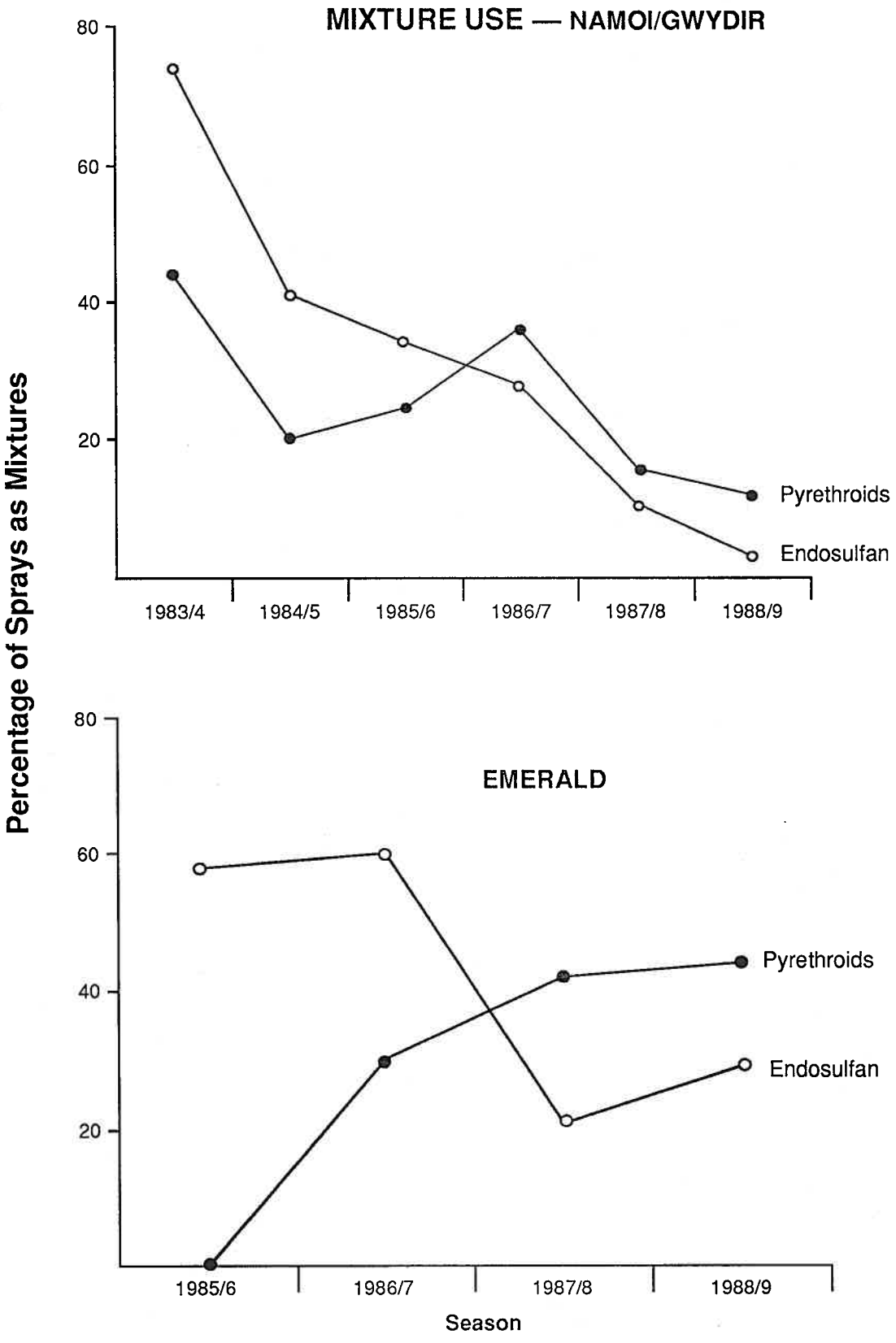


**Fig. A3.8** Use of mixtures with pyrethroids and endosulfan against *Heliothis* spp. in cotton in the Namoi & Gwydir river valleys of northern New South Wales and the Emerald Irrigation Area of central Queensland. Results expressed as the percentage of the total pyrethroid or endosulfan sprays applied as mixtures with either ovicides (eg. methomyl, chlordimeform) or with other larvicides or miticides (eg. parathion, monocrotophos, profenofos ,chlorpyrifos), as opposed to those applied alone.

## Cost of Insecticidal Control of *Heliothis* spp. in Cotton

| CHEMICAL GROUPING | INSECTICIDE | COMMONEST APPLICATION RATE (grams a.i. / hectare) | COST ($) / HECTARE | | | | |
|---|---|---|---|---|---|---|---|
| | | | 84/85 | 85/86 | 86/87 | 87/88 | 88/89 |
| Organochlorines | endosulfan | 720 | 9.60 | 11.91 | 14.07 | 14.70 | 13.50 |
| Organophosphates | profenofos | 750 | 29.70 | 32.43 | 26.79 | 29.76 | 31.71 |
| | chlorpyrifos | 750 | | 22.50 | 25.05 | 26.25 | 28.35 |
| | sulprofos | 1,008 | 26.60 | 27.94 | 35.38 | 35.48 | 35.48 |
| | parathion | 1,250 | 10.50 | 10.50 | 14.40 | 16.65 | 15.80 |
| Carbamates | methomyl | 450 | 17.00 | 23.80 | 29.00 | 29.84 | 29.90 |
| | thiodicarb | 750 | 25.04 | 25.04 | 24.50 | 50.00 | 50.00 |
| Pyrethroids | deltamethrin | 15 | 16.65 | 15.81 | 13.68 | 15.00 | 11.49 |
| | lambdacyhalothrin | 18 | | | 13.68 | 15.00 | 11.49 |
| | esfenvalerate | 25 | | | | | 11.50 |
| | alphacypermethrin | 40 | | | 13.68 | 15.00 | 11.50 |
| | cypermethrin | 84 | 16.38 | 15.54 | 14.18 | 14.99 | |
| | fenvalerate | 90 | 16.65 | 15.30 | 13.68 | 14.99 | |
| | fluvalinate | 105 | | | | | 25.34 |
| | Average pyrethroid cost / ha, excluding fluvalinate | | 16.69 | 15.72 | 13.77 | 15.00 | 11.50 |

**Table A3.1** Average cost of insecticides for the most commonly used rates for control of *Heliothis* spp. larvae in cotton (1984/85 to 1988/89 seasons). Costs are reported in $A per hectare for the average sized cotton farm (200-400 ha). Larger growers and corporate farms would obviously have access to lower prices.

# APPENDIX 4 - Pyrethroid & Endosulfan Resistance in *Heliothis punctigera* : Field and Laboratory studies

## Introduction

One of the most intriguing problems in Australian entomology has been the differential response of *Heliothis armigera* and *Heliothis punctigera* to insecticide selection pressure. Whereas *H. armigera* has developed resistance to virtually every insecticide used against it in any quantity (see Fig 1.1), there has not been any recorded resistance to any insecticide in the sibling *H. punctigera* species. This is quite extraordinary as the two species are co-incident on many crop hosts which are subjected to intense insecticidal protection especially cotton, tobacco and many broadleaf vegetable crops. In addition, whenever studied, the two species have consistently shown similar potential for metabolic detoxification of xenobiotics e.g. monooxygenases (Collins & Hooper 1984a) and DDT dehydrochlorinase (Sucksoong 1979). Thus it would seem that biochemically at least, both species are capable of developing resistance and that some other factor/s must be operating.

The most common explanation put forward for this differential response has been that the pool of unsprayed *H. punctigera* is so vast, that the treated proportion of the total population is only trivial. This would mean that any resistance genes would be swamped by the susceptible refugia and that *H. puntigera* would effectively manage its own resistance. This of course, is an ideal natural Insecticide Resistance Management (IRM) Strategy, even though it may mean that the IRM benefits may be offset by higher pest pressure. If this ecological explanation is correct, then theoretically, it should be possible to detect resistance genes, albeit at low frequencies, in the intensively sprayed crop areas. Furthermore, it should be possible to breed from these survivors and intensify the resistance levels by further selection in a closed laboratory colony.

The monitoring programme for evaluating insecticide resistance in *H. armigera* has already been described in Chapter 2. The sampling technique used in that study, resulted in a large number of the "contaminant" *H. punctigera* species in addition to the desired *H. armigera* species. As these superfluous *H. punctigera* were normally discarded after speciation, it was decided to exploit this ready supply of field collected *H. punctigera* to investigate the above ecological explanation for the differential response of the two *Heliothis* spp. to insecticide selection pressure.

## Methods & Materials

### *Field Screens*

The sampling procedure, areas and processing were as described in Chapter 2. However, in order to maximize the chance of detecting resistance, only the intensively sprayed cotton growing sites were sampled (that is, Emerald and the Namoi/Gwydir). The *Heliothis punctigera* larvae were reared to 30-40 mg and screened as either 3rd or 4th instars with the fenvalerate (0.05 µg/larva) or endosulfan (2.5 µg/larva) discriminating dose, as indicated in Appendix 2. The screening results were pooled for each Stage of the Resistance Management Strategy and expressed as the percentage of larvae surviving the discriminating dose plus the upper 95% confidence limit based on the pooled binomial standard error (see Table 2.1). The number of larvae tested in each Stage varied according

to the abundance of *H. punctigera* (less abundant later in the season) and the workload associated with the higher priority *H. armigera* component of the programme. When *H. punctigera* were abundant (mainly Stage 1), only a random subsample was screened. No attempt was made to calibrate a 'twin' discriminating dose technique as mentioned for *H. armigera* in Chapter 2, so larvae greater than 40 mg were discarded. The actual numbers tested in each Stage (up to 1,300/Stage) are given in Fig A4.1. The fenvalerate screens were initiated in mid and late 1986/87 in the Namoi/Gwydir and Emerald areas, respectively and in early 1987/88 for both areas for endosulfan. The *H. punctigera* screening was terminated at the end of the 1988/89 season in both sampling areas.

### *Laboratory Studies*

The survivors of the pyrethroid discriminating dose were pooled for both areas from the 1987/88 season onwards. This colony was further selected in the laboratory, initially at 0.05 µg but increasing gradually to 0.5 µg/30-40 mg 4th instar larva. Third instar larvae were excluded from these screens and subsequent bioassays to minimize the possible variability due to fluctuating metabolic detoxification capacity during the moulting cycle (Wilkinson & Brattsten 1972, Wilkinson 1983, Hodgson 1985, Collins & Hooper 1984b). This screening process continued for approximately two and a half years (20 generations) with fresh field material (fenvalerate discriminating dose survivors) being added to the colony as and when available, until the end of 1988/89 season. A comparable colony of endosulfan discriminating dose survivors was also maintained but was abandoned in late 1988 after no response to 12 months of selection at the 2.5 µg discriminating dose.

Once pyrethroid resistance levels stabilised, bioassays were performed against a range of pyrethroid structures including phenoxybenzyl alcohols with phenylacetic or cyclopropanecarboxylic acids and the resistance breaking simple benzyl alcohol pyrethroid, Series Two (see Chapter 10 for structures, sources etc). The synergists piperonyl butoxide (Pbo) and (S,S,S, -tributyl phosphorothioate (Def) were used in set amounts (50 and 20 µg/30-40 mg 4th instar larva, respectively), to determine the contribution of polysubstrate monooxygenase (PSMO) or esterase mediated resistance mechanisms, respectively. The maximum sublethal dose of each synergist was applied as indicated in Appendix 2, 5-15 minutes prior to the insecticide dose. The various pyrethroid structures ( ± synergists) were bioassayed against both the selected pyrethroid resistant colony and a pyrethroid susceptible field strain. Resistance factors were calculated ($LD_{50}$ resistant strain ÷ $LD_{50}$ susceptible strain) and were considered to be significant if there was no overlap of the 95% confidence intervals. The pyrethroid resistant strain was also tested with DDT and compared to two susceptible field strains. At least 48 larvae were tested at each dose within a 0-100% mortality range. Log dose probit lines were analysed using the Genstat statistical package.

## Results

### *Pyrethroid resistance*

The pooled Stage survival at the discriminating dose varied from less than 1% to just over 5%. The expected average survival was 0.9% with an upper 99% confidence limit of 2.1% (from Table A2.6). There were 5 occasions when the actual survival at the discriminating dose was significantly

greater than the expected upper 99% confidence limit (Fig A4.1). Four of these occurred in the Stage 2 pyrethroid window in both of the study areas in consecutive seasons.

Resistance levels to the phenoxybenzyl alcohol pyrethroids varied from 17.4x (deltamethrin) to 2.5x (cycloprothrin). There was no clear trend to higher resistance to the aromatic acid phenoxybenzyls (fenvalerate, fluvalinate, flucythrinate, cycloprothrin) compared to the aliphatic acid phenoxybenzyls (deltamethrin, cypermethrin, cyhalothrin) as was shown for pyrethroid resistant *Heliothis armigera* (see Chapter 10). However, the simple benzyl alcohol pyrethroid (Series Two) was able to fully overcome pyrethroid resistance in this species, as it does in *H. armigera* (see Chapter 10). The synergists Def and Pbo gave partial and full suppression of pyrethroid resistance, respectively (Table A4.1). However, early on in the colony's life, Pbo did not give full suppression (data not shown) so it is quite possible that an additional Pbo insensitive resistance mechanism/s may have been lost during culturing.

The pyrethroid resistant strain ($LD_{50}$ 1.07 µg/30-40 mg larva, 95% confidence interval 0.90 - 1.26, slope 2.8) was only 1.6x resistant to DDT (susceptible strain $LD_{50}$s 0.60 & 0.67 µg/larva, 95% confidence intervals 0.53 - 0.69 & 0.57 - 0.78, slopes 3.8 & 3.3, for DDT susceptible Emerald and Narrabri strains, respectively).

### *Endosulfan resistance*

The pooled Stage survival at the discriminating dose varied from less than 1% to just over 4%. the expected average survival was 4.0% with an upper 99% confidence limit of 8.2% (from Table A2.6). However, on no occasion did the actual survival at the discriminating dose exceed the expected upper 99% confidence limit.

The endosulfan selected laboratory colony $LD_{50}$ started at 0.52 µg/30-40 mg larva (95% confidence interval 0.45 - 0.61, Slope 3.1), peaked at 1.41 (95% confidence interval 1.19 - 1.68, Slope 2.5) and dropped to 0.77 (95% confidence interval 0.66 - 0.90, Slope 3.1) by the end of the 12 month selection period. Working off an average *Heliothis punctigera* susceptible $LD_{50}$ of 0.69 µg/larva (range 0.37 up to 0.97) (data from Table A2.6), it is clear that continued laboratory selection (at 2.5 µg/30-40 mg larva) could not increase endosulfan resistance above a 2.0x vigour tolerance level.

## Discussion

Pyrethroid resistance was detected in field populations of *Heliothis punctigera* at low frequencies (usually <5%). It is significant that the majority of these occasions occurred within the Stage 2 window. It was shown in Chapter 2 that the immediate increase in pyrethroid resistance in *H. armigera* during this period, was due to the selection of resistant adults. The eggs laid by these surviving resistant moths are then sampled and the selection pressure is immediately manifested as a sharp jump in resistance frequency. Although not specifically documented in this study, it is not unreasonable to assume that *H. punctigera* moths can also be selected for pyrethroid resistance. This would mean that pyrethroid resistance detected in *H. punctigera* within the Stage 2 window, would be due to selection of a local adult population. This would be the only time in the season when the

impact of selection pressure would be manifested locally. Pyrethroid selection of larvae would be manifested in the following generation in Stage 3 and therefore subject to the confounding influence of dilution from susceptibles immigrating from the unsprayed refugia. This is particularly so for the obligate migrant *H. punctigera* which is much more mobile than the facultative migrant *H. armigera* (Zalucki et al. 1986) In fact, because of its greater mobility, wider host range and complex diapause strategy, Fitt et al. (1989) suggest that the composition of *H. punctigera* populations may include elements of recent local origin, locally produced individuals which had entered diapause some time before and long distance migrants whose numbers are affected by seasonal climatic patterns far from the cropping areas. Thus it is no surprise, that this complex mixing pattern and dilution from numerically superior susceptibles, would mask any detectable resistance in Stages 1 and 3. However, no such problem should occur in Stage 2 where selection pressure on adults can be immediately detected. Indeed, this was the case in this study, where low but significant levels of pyrethroid resistance were found within the Stage 2 window in both of the intensively sprayed cotton areas in consecutive seasons.

Closed laboratory selection of these survivors indicated clearly that *H. punctigera* can express moderate resistance to the range of currently commercially available pyrethroid structures. It would seem that the dominant resistance mechanism is metabolic detoxification mediated by monooxygenases. The partial synergism by Def could indicate some esterase activity as well but as mentioned in Chapter 9, Def is not a specific esterase inhibitor and can inhibit oxidases as well. Thus the partial synergism by Def could simply indicate imperfect inhibition of the monooxygenase resistance mechanism without necessarily any esterase role. Early in the life of the culture, Pbo did not give full suppression. This could have been due to a low level residual nerve insensitivity mechanism or a reduced penetration factor or both, as was found in pyrethroid resistant *H. armigera*. However, this Pbo insensitive resistance seemed to be lost during culturing and oxidative metabolic detoxification mechanisms dominated as indicated by the excellent suppression by Pbo, the full efficacy of the oxidative metabolic resistance breaking pyrethroid and the lack of cross resistance to DDT.

No detectable endosulfan resistance was found in any Stage. This could be due to either; there was no resistance to be found, or that the resistance frequencies were too low to be detected. The total number of *H. punctigera* screened with endosulfan was 8,041 which should be able to detect resistance frequencies down to $3.7 \times 10^{-4}$ with 5% error (calculated from Roush & Miller's (1986) rearranged formula given in Rosenheim & Hoy 1988). This is within the normally quoted range ($10^{-3}$ to $10^{-5}$) for the frequency of resistance genes in unselected wild populations (Georghiou 1983, Wood 1981). However, it cannot be concluded from this study that *H. punctigera* did not develop resistance to endosulfan, as the resistant phenotypes could well occur at lower frequencies.

The difference in detectability of pyrethroid and endosulfan resistance can probably be attributed to the greater importance of adult selection for pyrethroids. This, coupled fortuitously with a sampling procedure which can immediately detect this selection, avoids the confounding influence of population mixing and dilution in a highly migratory, polyphagous species.

Many authors have long recognised that some highly migratory pest species exploit a wide variety of non-economic hosts and are unlikely to be subject to the extreme selection pressure encountered by obligate pests subject to efficient centralised control (Wood & Bishop 1981, Tabashnik & Croft 1982). *Heliothis punctigera* would seem to fit well into this category. This polyphagous species has a wide range of predominantly (84%) unsprayed hosts (Table A4.2) and is highly migratory, being well adapted to the erratic distribution of rainfall and suitable hosts in inland Australia (Farrow & Daly 1987). On the other hand, the relatively oligophagous and facultatively migratory *H. armigera* species, seems less well adapted to the Australian scene. It has relatively few native hosts and a high proportion (29%) of sprayed host crops (Table A4.2). Bull and Menn (1990) suggest a similar scenario for the American *Heliothis* complex (*H. virescens* and *H. zea* being ecologically equivalent to *H. armigera* and *H. punctigera*, respectively).

**HOST PLANTS of *HELIOTHIS spp.***

| | | No. of Host Plants & (% of total) | |
|---|---|---|---|
| | | *Heliothis punctigera* | *Heliothis armigera* |
| Unsprayed | — Native | 30 (24%) | 8 (11%) |
| | — Naturalised weed / Garden | 36 | 21 |
| | — Crops | 41 | 24 |
| Sprayed | — Crops | 20 (16%) | 22 (29%) |
| TOTAL | | 127 | 75 |

**Table A4.2** Ecology of *Heliothis punctigera* and *Heliothis armigera* host plants (data derived from Zalucki et al. 1986 ).

The preceding information can help to explain the recidivist resistance nature of *H. armigera*. Australia has always figured prominently at the forefront of insecticide resistance problems in *Heliothis* (eg. DDT resistance in the Ord, pyrethroid resistance at Emerald). Some people suggest that this reflects poorly on the pest management practices of Australian summer crop growers. However, an unfortunate combination of a poorly adapted, possibly relatively recent immigrant in an ecosystem dominated by recurring droughts, has probably had a far greater impact on resistance development than putative poor pest management. The already relatively narrow range of hosts for *H. armigera* would be even further restricted to irrigated crop hosts during periods of drought. *Heliothis armigera* populations would be then concentrated on these high value irrigated crops such as cotton and would be subject to intense selection pressure with little opportunity for dilution. Thus, it is not surprising, that in a land where frequent droughts are the rule rather than the exception, that resistance in *H. armigera* has been an all too familiar problem.

The above situation with *H. armigera* contrasts sharply with that in *H. punctigera*. This difference clearly illustrates the value of maintaining an effectively large susceptible gene pool. One of the first workers to recognise this was Benson who in his remarkably avant-garde 1971 treatise on IRM, went so far as to suggest the large scale release of susceptible insects into the pest population even if it meant "sacrificing some of our food to the right insects, those with susceptible genotypes". He suggested that this "genetic infusion" technique would be the only ultimate long term solution to IRM as it "controlled the evolution of pest species". Perhaps the case of self regulated resistance management in *H. punctigera* described in this paper, is a living example of Benson's "ultimate IRM solution".

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## PYRETHROID RESISTANCE IN *HELIOTHIS PUNCTIGERA*

| Pyrethroid alone or + Synergist | RESISTANT Strain LD50 (95% Confidence Limits) | Slope | RF | SUSCEPTIBLE Strain LD50 (95% Confidence Limits) | Slope |
|---|---|---|---|---|---|
| Deltamethrin | 0.087 (0.074, 0.104) | 2.7 | 17.4 * | 0.005 (0.004, 0.006) | 2.8 |
| Fenvalerate | 0.116 (0.096, 0.141) | 2.4 | 10.5 * | 0.011 (0.009, 0.012) | 3.2 |
| Fluvalinate | 0.258 (0.214, 0.312) | 2.3 | 8.0 * | 0.032 (0.028, 0.038) | 3.3 |
| Flucythrinate | 0.111 (0.092, 0.133) | 2.4 | 6.5 * | 0.017 (0.014, 0.020) | 2.9 |
| Cypermethrin | 0.118 (0.095, 0.149) | 1.6 | 6.3 * | 0.019 (0.016, 0.022) | 3.4 |
| Cyhalothrin | 0.025 (0.019, 0.031) | 1.6 | 6.0 * | 0.004 (0.003, 0.005) | 3.0 |
| Cycloprothrin | 0.254 (0.213, 0.305) | 2.7 | 2.5 * | 0.101 (0.083, 0.129) | 2.2 |
| Series Two | 0.032 (0.026, 0.039) | 1.7 | 1.0 ns | 0.032 (0.028, 0.037) | 3.8 |
| Def 20 + Fenvalerate | 0.043 (0.034, 0.054) | 2.1 | 2.2 * | 0.019 (0.016, 0.023) | 2.3 |
| Pbo 50 + Fenvalerate | 0.014 (0.012, 0.016) | 2.9 | 1.1 ns | 0.013 (0.011, 0.016) | 3.2 |
| Pbo 50 + Cycloprothrin | 0.077 (0.067, 0.090) | 3.5 | 1.2 ns | 0.065 (0.055, 0.077) | 3.0 |
| Pbo 50 + Fluvalinate | 0.039 (0.033, 0.047) | 2.6 | 1.3 ns | 0.029 (0.025, 0.033) | 3.4 |

**Table A4.1** Pyrethroid resistance in a strain of *Heliothis punctigera* obtained initially from pooled survivors of the Emerald & Namoi/Gwydir 1987/88 & 1988/89 fenvalerate discriminating dose survivors (see Fig A4.1) and selected further in the laboratory with increasing fenvalerate doses (0.05-0.5 µg / 30-40 mg 4th instar larva)until resistance levels stabilised (approx $F_{20}$). Synergists (Def & Piperonyl butoxide {Pbo}, 20 & 50 µg / 30-40 mg 4th instar larva, respectively) were applied 5-15 minutes before the insecticide dose . $LD_{50}$ expressed in µg / 30-40 mg 4th instar larva. Resistance factors (RF) expressed as $LD_{50}$ resistant strain ÷ $LD_{50}$ susceptible strain. * , ns indicate non-overlap & overlap of susceptible & resistant 95% confidence intervals, respectively.

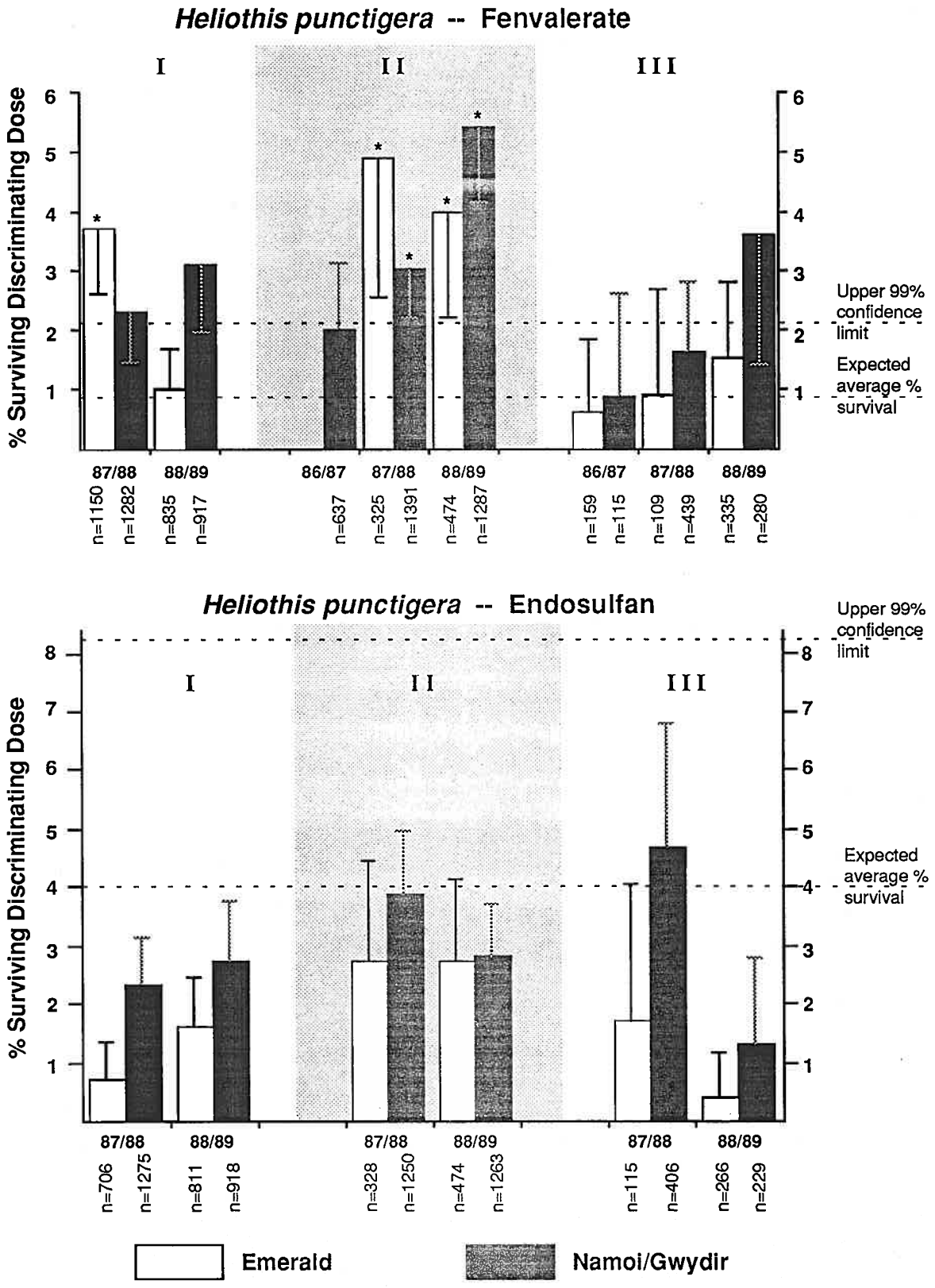


**Fig. A4.1** Survival of *Heliothis punctigera* at the fenvalerate and endosulfan discriminating doses (0.05 and 2.5 micrograms per 30-40mg larva respectively) at two sites (the Namoi and Gwydir river valleys of northern NSW and the Emerald Irrigation Area of central Queensland) for Stages I , II and III of the Resistance Management Strategy. Horizontal lines represent the average and upper 99% confidence limit for the expected survival at the discriminating doses of fenvalerate (0.9% and 2.1% respectively, calibrated on 10 susceptible strains) and endosulfan (4.0% and 8.2% respectively, calibrated on 11 susceptible strains) [data taken from Table A2.6]. Vertical bars indicate the upper 95% confidence limit (based on the pooled binomial standard error). * indicates % survival at the discriminating dose significantly greater ($p<0.05$) than the expected upper 99% confidence limit. n = the number of larvae tested in each Stage.