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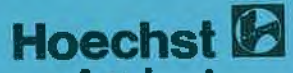

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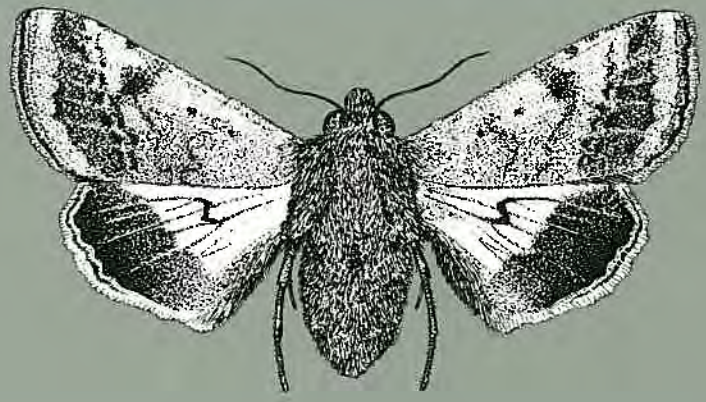
Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia

Neil W. Forrester, Matthew Cahill, Lisa J. Bird and Jacquelyn K. Layland



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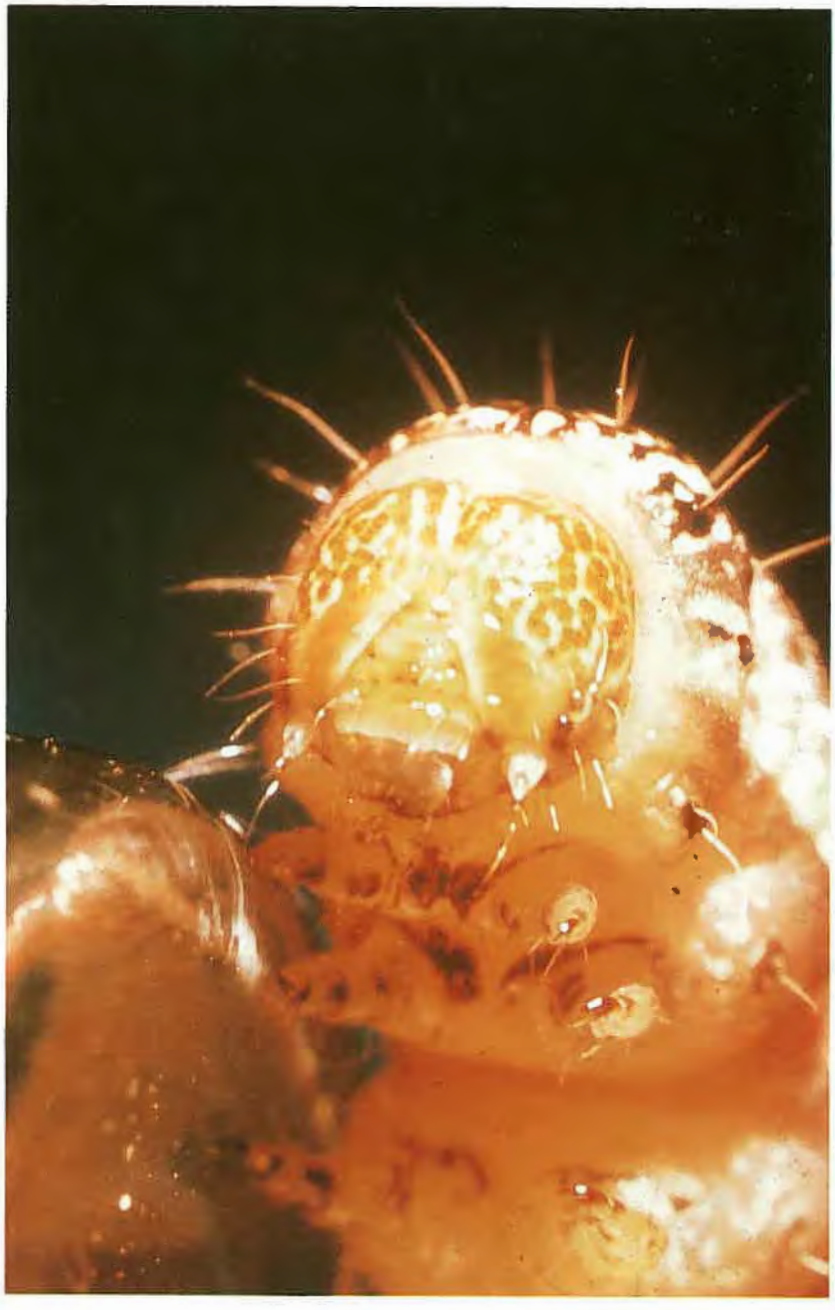

Helicoverpa armigera larva close-up. *New South Wales Agriculture.*

Helicoverpa armigera moth close-up. *New South Wales Agriculture.*

Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) 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' (Taylor 1986). His prophetic comment remains as pertinent now as it was 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.

Neil W. Forrester

Abstract

An insecticide resistance management (IRM) strategy was introduced into the summer rainfall cropping areas of eastern Australia in the 1983/84 season. The aims of this IRM strategy were to manage pyrethroid and endosulfan resistance problems in *Helicoverpa armigera* (Hübner), formerly *Heliothis armigera* (Hübner), and to avoid any possible future problems with organophosphate or carbamate resistance. 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 incorporated into an acceptable integrated pest management (IPM) programme. The voluntary restrictions were applied to all crops susceptible to *H. armigera*. They were even applied to other co-incident pest species on these hosts, such as sorghum midge *Contarinia sorghicola* (Coquillet) (Diptera: Cecidomyiidae), as it was shown that pyrethroids applied to flowering sorghum for midge control caused selective mortality of co-incident *H. armigera* larvae and resulted in differential selection for resistance. The demonstration of the independence of the endosulfan and pyrethroid resistance mechanisms vindicated the sequential use of these two groups in Stages I and II of the IRM strategy, respectively.

The impact of the IRM strategy on pyrethroid and endosulfan resistance was followed using a monitoring technique based on discriminating dose testing of larvae reared from field collected eggs. This proved to be a very sensitive technique which facilitated fine tuning of the strategy's guidelines as and when necessary, e.g. the reduction of the pyrethroid window from 42 to 35 days from the 1989/90 season onwards. Pyrethroids selected for resistance in both moths and larvae and this was manifested within the Stage II window and in the early Stage III period, respectively. The two main factors influencing the frequency of pyrethroid resistance were dilution by susceptibles immigrating from the refugia and pyrethroid selection pressure. However, as the refugia became increasingly contaminated, their effectiveness as a dilution source declined, resulting in gradually increasing pyrethroid resistance levels in all areas over time. This highlights the importance of maintaining an effectively large susceptible gene pool for sustained dilution of resistance as was shown in the case of self regulated resistance management in the polyphagous, highly mobile sibling species *Helicoverpa punctigera* (Wallengren). Inadequate cultivation of post-harvest fields harbouring overwintering pupae resulted in the carry over of large numbers of resistant pupae. The strategy was shown to be a successful delaying tactic for pyrethroid resistance. The possible reasons for the much more successful management of endosulfan resistance are discussed.

The Via tolerance curve analysis of F1 data indicated an abrupt change in the relative importance of field pyrethroid resistance mechanisms following the introduction of the IRM strategy. The strategy favoured the selection of the more amenable oxidative resistance mechanism over the intractable nerve insensitivity resistance mechanism which was also clearly demonstrated by the dual insecticide ± synergist discriminating dose technique. Two possibly complementary explanations are put forward for this; differential genetic dominance, and/or selection in more than one life stage. The Beeman-Nanis analysis was unsuccessful in identifying the relative importance of the field resistance genes due to lack of full genetic dominance.

Moths expressed pyrethroid resistance to both direct (eye test) and indirect (tarsal plate test) exposure without hypo-irritability. Selection of adults in the field resulted in dispersal of resis-

tant moths from sprayed to nearby unsprayed cotton. The phenotypic expression of pyrethroid resistance in moths declined with age. This resulted in poor correlation of adult resistance (determined from pheromone trapped males) and resistance in field collected eggs. Oxidative metabolic detoxification was shown to be the major pyrethroid resistance mechanism in moths as well as larvae.

Both pyrethroid and endosulfan resistant *H. armigera* larvae were shown to have marginally longer development times. However, these were not manifested as significant biological deficits in laboratory and field competition studies on pyrethroid resistant larvae and prepupae (endosulfan not studied). The absence of back selection against pyrethroid resistant *H. armigera* in the unsprayed refugia, due to the lack of any selective disadvantage in the immature stages (adults not studied), helps explain the gradually deteriorating pyrethroid resistance situation.There was no evidence of selection of fitness modifiers to overcome the slower development of either pyrethroid or endosulfan resistant larvae.

The demonstration that the strategy has favoured selection of the more amenable oxidative resistance mechanism, led to the study of possible chemical countermeasures, such as synergists and resistance breaking pyrethroids. The methylenedioxyphenyl and acetylenic compounds were the most effective synergists with moderate activity from some organophosphate compounds. All the other compounds tested were either ineffective or only marginally effective, including most organophosphates tested, pyrethroid analogues, N-alkyls, esterase and glutathione transferase inhibitors, various nitrogen heterocycles, juvenile hormone and analogues, formamidines, organochlorines, anti-oxidants, and kojic acid. The most promising synergists indicated for further evaluation were synthetic analogues of piperonyl butoxide (Pbo), phosmet, propargite and possibly also fenthion, phosalone, azinphos-ethyl, pyrazophos and kinoprene. Studies with various solvents indicated that the mode of action of Pbo and the other synergists in this study, is principally true biochemical inhibition and not quasi-synergism (improved penetration). Studies on Pbo indicated that a set rate of Pbo should be used, irrespective of the activity of the accompanying pyrethroid, residual activity of Pbo is poor but that this could be partially overcome by increasing the rate, straight Pbo applied onto a weathered pyrethroid deposit could restore control but only temporarily and would probably be of little practical field use and that, no difference in residual activity could be found between the four Pbo formulations tested. In order to preserve the long-term effectiveness of Pbo as a pyrethroid synergist within the Australian IRM strategy, an optimal use strategy (based on synergist rotation within the present insecticide rotation scheme) is discussed.

The structural requirements for designing a resistance breaking pyrethroid to overcome oxidative metabolic pyrethroid resistance in *H. armigera* were studied. Changes from the conventional phenoxybenzyl alcohol moiety could overcome most, if not all, resistance. Simple benzyl alcohols were the most effective followed by cyclopentenolones and a methylated biphenyl alcohol. Incorporation of synergophore groupings (methylenedioxyphenyl and acetylenic) were fully effective in breaking resistance. Changes from the conventional central ester bond to an ether and reversion to an unsubstituted alpha carbon analogue, both lowered resistance. Some evidence was found to indicate that Pbo could be acting both as a classical monooxygenase inhibitor and a preferential penetration synergist in resistant larvae. Fully or partially resolved isomers were clearly much more toxic on resistant strains, indicating a possible blocking effect of the inactive isomers. A simple benzyl resistance breaking pyrethroid (Series Two) was shown to be equally effective on both adult and larval *H. armigera*, giving similar results to a pyrethroid/Pbo mix. The ideal requirements for a resistance breaking pyrethroid are discussed as well as factors acting against their possible commercialization.

Section 1

The Australian insecticide resistance management strategy

Summary

In response to field pyrethroid failures against *Helicoverpa 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 *Helicoverpa armigera* susceptible crops (including cereals, oilseeds, grain legumes, tomatoes, tobacco and cotton) and even to other co-incident pest species. From its inception, compliance with the voluntary strategy has been exceptional.

Introduction

In January 1983, pyrethroids failed to give satisfactory field control of *Helicoverpa armigera* (Hübner), formerly *Heliothis 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 1970s, they had many benefits over what was then available. They were very cost-effective at extraordinarily low dosage 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, 1985a). In fact, by 1986, their popularity was such that they accounted for around 25% of all insecticides used in agriculture and public health (Jackson, 1989; Hirano, 1989a). They were particularly favoured in cotton because of their contact mode of action and good efficacy against previously resistant pests and by the mid-1980s accounted for 49% of the world cotton insecticides market (Riley, 1989; Watkinson, 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 six 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, 1990a).

Background, format and aims of the Australian strategy

Insecticide resistance has been a recurring problem for Australian summer crop, particularly cotton, growers. *Helicoverpa armigera* has developed resistance to virtually every insecticide group used against it, including the organochlorines, cyclodienes, organophosphates, carbamates and pyrethroids (fig. 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. 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 one of the four to five 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.

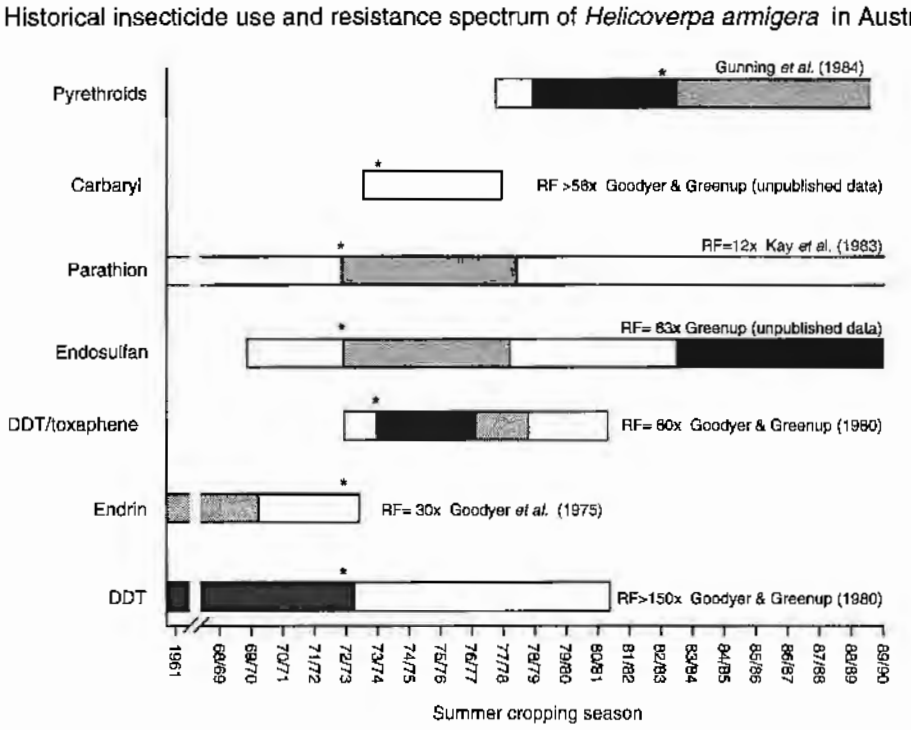


Fig. 1. Historical sequence of insecticide use in Australian cotton (first significant commercial crop 1961) and the development of resistance in the recidivist cotton and field crop pest *Helicoverpa armigera*. Horizontal bars indicate the duration and intensity of use for each insecticide (low ▭, moderate ▬, high ■). *Indicates the first records of field resistance. RFs indicate the maximum recorded resistance factors.

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 three of the four to five 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 was also expected to be used principally early in the 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 or chlordimeform) to pyrethroids and/or endosulfan under high egg pressure. 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 only be used in these Stages as and when necessary (e.g. for control of co-incident mites and *Helicoverpa* 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.*, 1987b) for the management of organophosphate and carbamate resistance, compared to the more restrictive curative rotation approach for pyrethroids and endosulfan.

Because of *H. armigera's* polyphagous nature, it was recommended that all growers of susceptible host 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 for *H. armigera* in susceptible crops also applies to other insect pests in

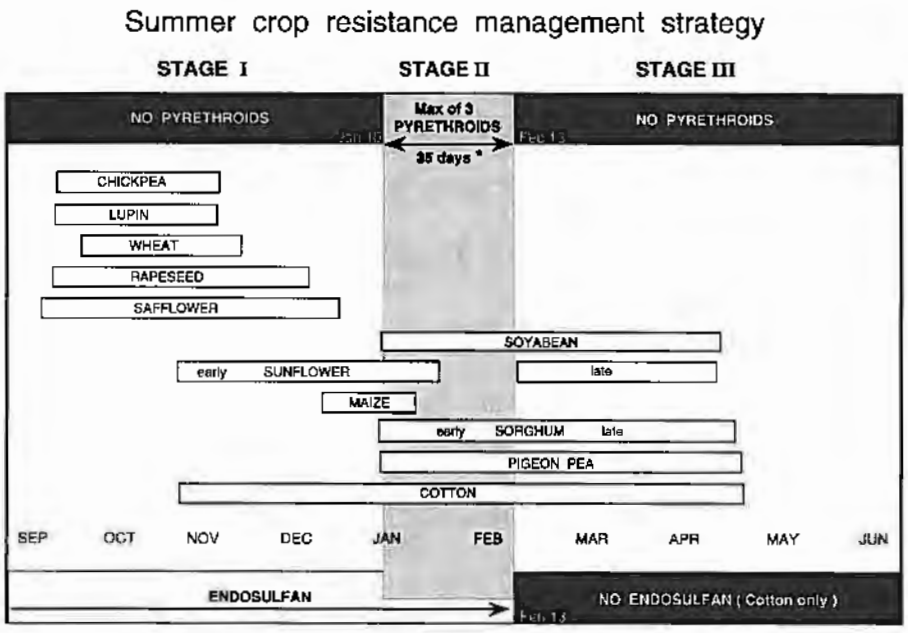


Fig. 2. Summer crop resistance management strategy for northern New South Wales and southern Queensland (the Emerald irrigation area of central Queensland has an earlier Stage II window, beginning 1st Jan and finishing 3rd Feb). Crop intervals indicate the periods when control of *Helicoverpa* spp. (or 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 (10th Jan–20th Feb); 1989/90 onwards, reduced to 35 days.

these crops, such as sorghum midge (*Contarinia sorghicola* (Coquillet) (Diptera: Cecidomyiidae)), armyworms (*Mythimna convecta* (Walker) (Lepidoptera: Noctuidae)) and *Nysius* spp. bugs (Hemiptera: Lygaeidae), which are often present with *H. armigera*. It was suggested that spraying of these pests with pyrethroids would also select incidentally for resistance in *H. armigera* (see Section 6). Because of the multicrop nature of the strategy, the timing of the pyrethroid window was designed to satisfy, as far as possible, the needs for insect control in each crop. Thus, in cotton, it was aimed at peak flowering/early boll set, a vulnerable period of the cotton growth cycle when access to the highly efficacious contact pyrethroids would be most appreciated by cotton growers. 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 need).

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 in the 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 parasitoids and predators and also to avoid the potential upsurge 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 in the 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* (see Section 7).
- 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 Section 7).
- Sample over-wintering pupae under cotton stubble and cultivate should they exceed threshold (Fitt & Forrester, 1987).
- Target pyrethroids to egg hatch, to avoid selection of older established larvae (Daly *et al.*, 1988a).
- Check crops frequently and thoroughly and spray on threshold. This can minimize the need for sprays and ensure their maximum effective-

ness through optimum timing (especially important for the shorter-residual organophosphates).
- Utilize host-plant resistance wherever possible (e.g. okra leaf varieties offer some degree of control, particularly for mites).
- If a pyrethroid is used to control *C. sorghicola*, do not follow up with a pyrethroid for *H. armigera* control, as the midge spray will have already selected for pyrethroid resistant *H. armigera* (see Section 6).

This integrated approach was designed to vary mortality factors so that selection pressure would not be channelled to any one control measure.

Discussion

Insecticide usage surveys (see Appendix 3) have indicated that there has been a universal adoption of the strategy 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 growers were well aware of the economic consequences of uncontrolled resistance. They had experience of DDT and DDT/toxaphene resistance in the early 1970s, particularly in the Ord (Hearn, 1975) and endosulfan resistance in the mid 1970s. They understood that if countermeasures were not taken 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 (e.g. Hearn, 1975; Vaughan & Leon, 1976; Bottrell & Adkisson, 1977; Dover & Croft, 1984; National Research Council, 1986; Matthews, 1989). 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 three different sites of action (Hammock & Soderlund, 1986), has been adopted in practice.

In their tome on resistance management, the National Research Council (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 (e.g. a selection interval of one generation was allowed, followed by a regression interval of three to four 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 recognize these genuine efforts to legitimize 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). Pyrethroids and endosulfan are the two most widely used insecticide groups used in Australian summer field crops, accounting for over 80% of insecticide use against *Helicoverpa* spp. So their loss through resistance would have a major economic impact, particularly in cotton. The next two sections evaluate the effectiveness of the Australian IRM strategy in managing resistance to these two key insecticides.

Section 2

Evaluation of the impact of the strategy on pyrethroid and endosulfan resistance: discriminating dose studies

Summary

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 procedures. This has resulted in the maintenance of the strategy's excellent compliance rate.

Pyrethroids imposed selection for resistance in both moths and larvae, resulting in increases in resistance within the Stage II window and the early Stage III period, respectively. These two peaks effectively merged into one large peak while the period of pyrethroid use 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 appear to be dilution by susceptibles immigrating from the refugia, followed by pyrethroid selection pressure. However, as the refugia became increasingly contaminated, their 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 crop Emerald study area because of premating selection. This, along with the higher *Helicoverpa armigera* pressure at Emerald, probably offset any potential benefit of the longer crop season at this site. Inadequate cultivation of post-harvest fields harbouring overwintering pupae, presumably due to low price forecasts in the economically sensitive cotton industry, resulted in the survival 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 proved 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 lower 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 recognized for some time (Cook, 1981; Dover & Croft, 1986; Georghiou & Taylor, 1986; Hammock & Soderlund, 1986; National Research Council, 1986; Dennehy, 1987). Indeed, Dennehy (1987) considered that 'monitoring methodology was the vehicle needed to make most Resistance Management Strategies implementable and verifiable'. This need was also recognized 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 Section 3).

Previous resistance studies have evaluated discriminating doses on field material (e.g. Georghiou & Taylor, 1976; Pree & Wagner, 1987) or on F1 progeny of field material (e.g. Roulston *et al.*, 1981; Denholm *et al.*, 1983). Some studies have even attempted to correlate operational and biological factors with changes in resistance (e.g. Georghiou *et al.*, 1973; Wolfe & Barrett, 1986). However, none were designed specifically to monitor the impact of a resistance management strategy on a continuous, 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 & Bishop (1981) recognized that the '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 and materials

Sampling areas (fig. 3)

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 large monocultures of irrigated cotton, together averaging 50,000-60,000 hectares of cotton per season (fig. 52). 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 crop area capable of growing up to 12,000 irrigated hectares of various crops, but mainly cotton (table 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 crop season than the Namoi/Gwydir area. For example, while *Helicoverpa armigera* has only four to five generations per season in the Namoi/Gwydir area, it can have up to six to seven at Emerald. Therefore sampling at Emerald usually started earlier and finished later (September-May) than in the Namoi/Gwydir area (November-April). While the first two sites chosen (Namoi/Gwydir and Emerald) were intensively sprayed cotton areas, the third was an essentially unsprayed refugium 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 km 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 organizing an intensive monitoring programme at this relatively remote site (some 1000 km from the central Narrabri laboratory). Because

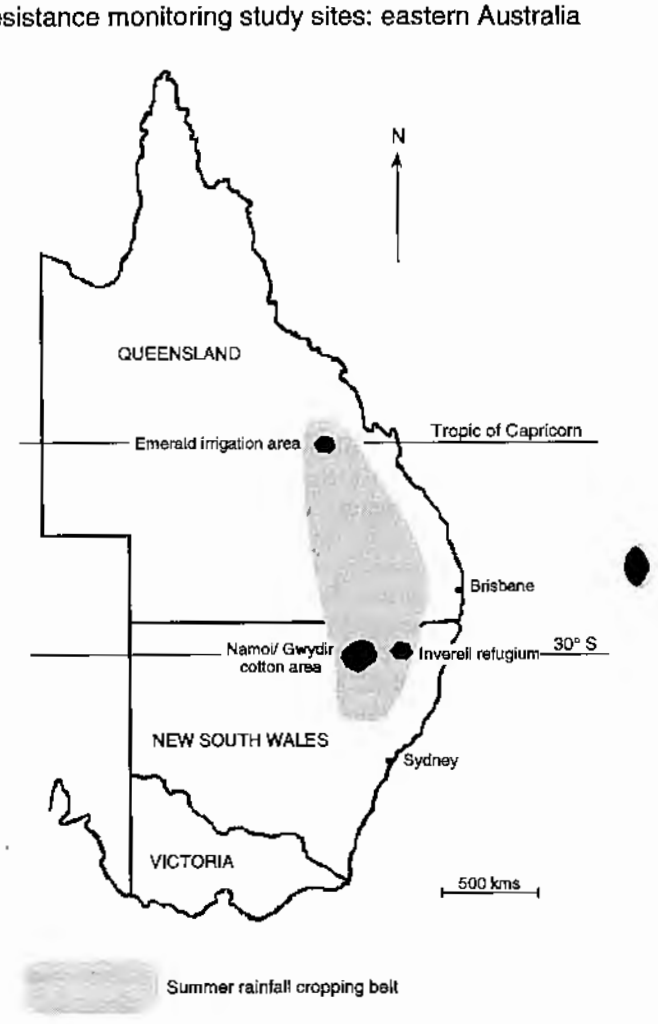


Fig. 3. Location of the three resistance monitoring study sites within the eastern Australia summer rainfall cropping zone; the Emerald irrigation area of central Queensland (an irrigated mixed cropping system, mainly cotton), the Namoi and Gwydir river valleys of northern New South Wales (an irrigated cotton monoculture) and an unsprayed refugium area of dryland (raingrown) alternative host crops (mainly maize, sorghum and sunflowers), centred just west of Inverell in northern New South Wales.

of the presumed importance of the refugia as a source of susceptibles for dilution of resistance, an example of the refugia (the Inverell area) was incorporated into the programme from 1987/88 onwards.

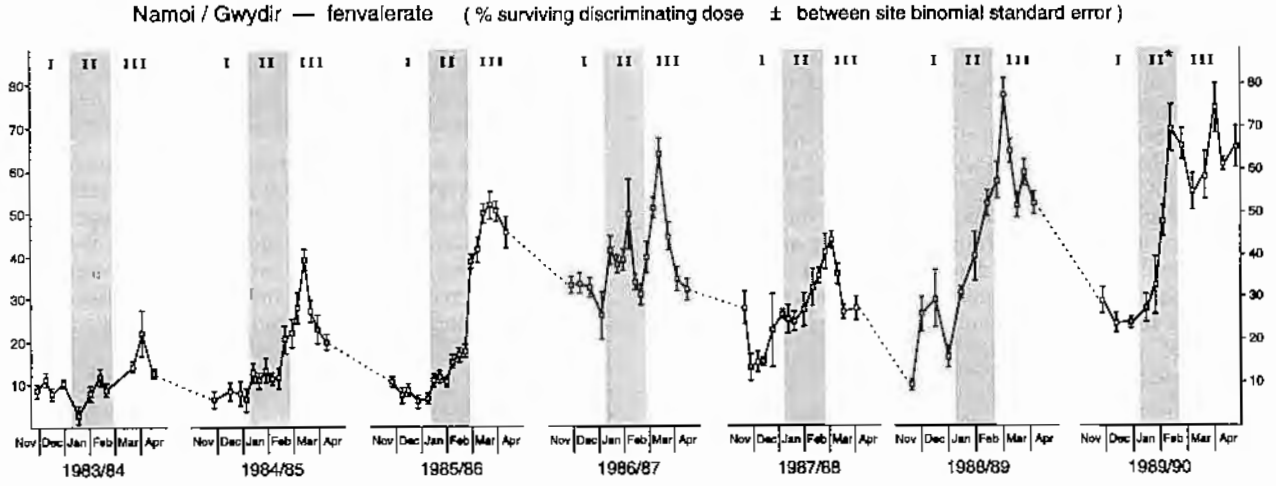
The same sampling areas have been used each season and collection sites within each study area were chosen randomly. A conscious effort was made to spread the collection sites evenly throughout each study area and to avoid concentrating on resistance 'hot spots', or including samples from outside the originally chosen areas as this was a potential source of bias (e.g. Plapp *et al.*, 1990b).

Sampling procedure

Each property was considered as a basic sampling unit. Eggs (mixtures of both *H. armigera* and *H. punctigera*, in unknown proportions) were collected at random from as many fields as possible at 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. It was hoped that 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.

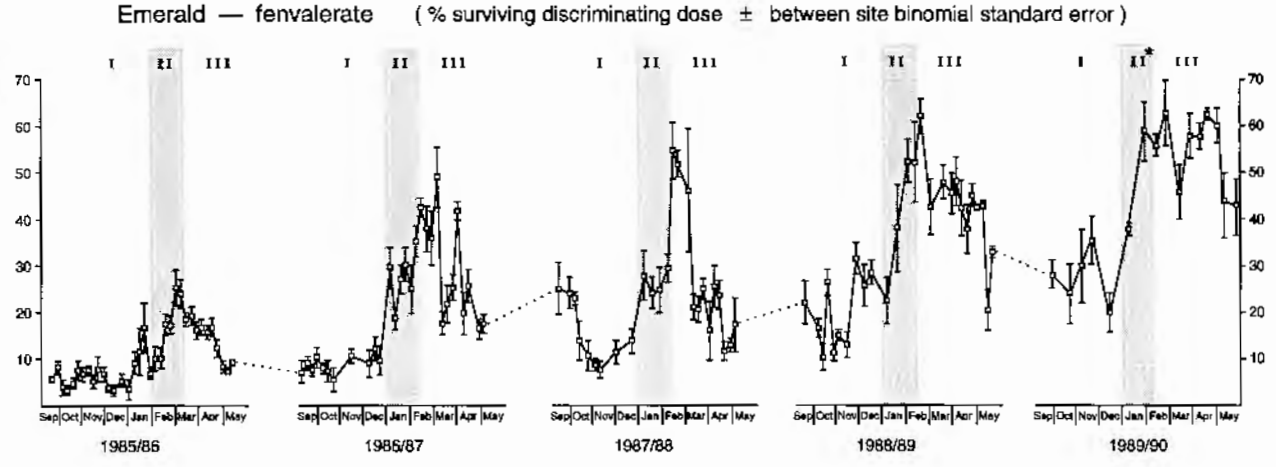
Sample processing

On their receipt in the laboratory, eggs were removed from 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,



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

Fig. 4. Weekly pyrethroid resistance in *Helicoverpa armigera* from the Namoi and Gwydir river valleys of northern New South Wales for the seven 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 µg per 30–40 mg larva) ± between site binomial standard error.



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

Fig. 5. Weekly pyrethroid resistance in *Helicoverpa armigera* from the Emerald irrigation area of central Queensland for the past five 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 mg per 30–40 mg larva) ± between site binomial standard error.

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°C ± 2°C but samples from the remote Emerald site were held at 12°C and despatched at weekly intervals in insulated transportable coolers, to the central testing laboratory at Narrabri. Samples from Emerald arrived usually in two to three days, but as neonates had access to artificial diet, hatching in transit posed no difficulties.

Hatched larvae were identified to species at the second or third instar to either *H. armigera* or the susceptible sibling species *H. 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 third or fourth 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 maximize the yield of suitably sized testing larvae. Late third instar moulting larvae were avoided and were held overnight at 18°C and tested the next day as either 30-40 mg or 40-60 mg fourth instars with the appropriate fenvalerate discriminating dose (either 0.2 or 0.5 µg/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 I when *H. armigera* numbers were at their lowest.

In the 1986/87 season, a second insecticide (endosulfan) was incorporated into the field screening programme. Starting with that season, each sample of *H. armigera* larvae was split equally and randomly into two subsamples and tested with either the fenvalerate discriminating dose (as previously) or the endosulfan discriminating dose (10 µg 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 screening test). 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

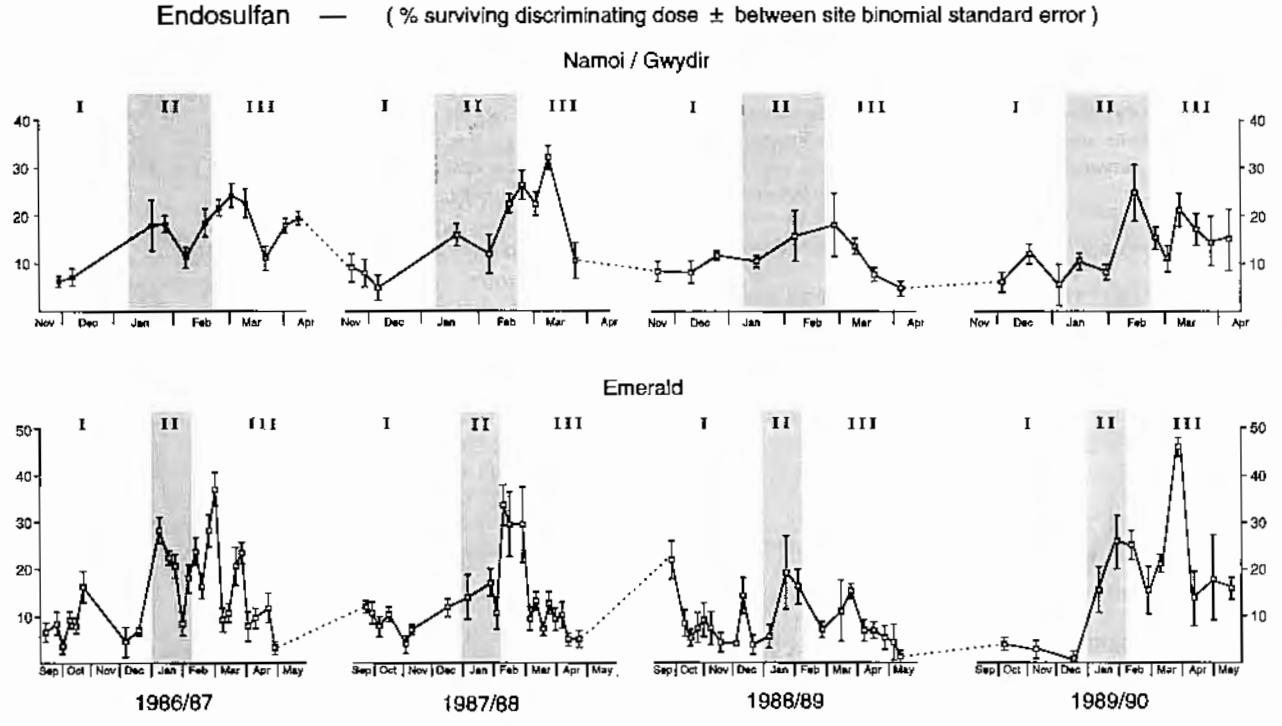


Fig. 6. Weekly endosulfan resistance in *Helicoverpa armigera* from the Namoi and Gwydir river valleys of northern New South Wales and the Emerald irrigation area of central Queensland for the past four seasons of the Resistance Management Strategy (for Stages I, II and III). Results expressed as the percentage of larvae (reared from field collected eggs) surviving the endosulfan discriminating dose (10 µg per 30–40 mg larva) ± between site binomial standard error.

Helicoverpa armigera larva in cotton boll.
New South Wales Agriculture.

Topical application to *Helicoverpa armigera* larvae in modified tissue culture trays. *New South Wales Agriculture.*

Chickpeas, a favoured host plant for *Helicoverpa armigera,* direct drilled into slashed cotton stubble. *New South Wales Agriculture.*

Collecting eggs off cotton for resistance testing.
New South Wales Agriculture.

then either pooled into collecting weeks and graphed using between site binomial standard error estimates (figs 4, 5 and 6, table 1), or into collecting Stages (I, II or III) and tabled or graphed using standard errors of the mean (table 2, fig. 7). Comparisons were also made between the weekly pooled and between site binomial standard errors (table 1), as suggested in Sawicki *et al.* (1989). The significance of the indices of total season selection pressure (increase in pyrethroid resistance between Stages I and III, table 5), inter-season decline (decrease in pyrethroid resistance between previous Stage III and the following Stage I, table 4) and adult selection (increase in pyrethroid resistance between Stages I and II, table 3), were all made using unpaired t tests on combined Stage means.

Rainfall and crop surveys

Rainfall records for 45 sites spread throughout the New South Wales portion of the eastern Australia summer rainfall crop belt (fig. 3), 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. 8). This time period was chosen as rainfall in these months would impact on the growth of sorghum, the major alternative host flowering during Stage II 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. 8). This allowed a better assessment for the Stage III dilution potential of the surrounding refugium 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) alternative host.

Winter cultivation surveys

After recognizing 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 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 *H. armigera* (table 6).

Results

Species composition (figs 9 and 10)

Namoi/Gwydir *H. armigera* Stage I 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 I *H. armigera* levels at Emerald were quite variable from season to season, probably reflecting changing crop cultivation patterns in response to variable rainfall. However, it is quite clear that Stage I *H. armigera* pressure at Emerald is significantly higher than in the Namoi/Gwydir. Stage II *H. armigera* levels, both in the Namoi/Gwydir and Emerald, were generally higher than in Stage I, particularly towards the end of this period. The changeover to late season *H. armigera* dominance was generally complete in Stage III 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 I and II (tables 2 and 3) and sharp peaks in Stage III (fig. 4), except in the 1989/90 shortened pyrethroid window season. Normally resistance peaked in the first few weeks of Stage III (early March), but in the 1989/90 season it in fact dropped during this period, reaching a peak only in late March. This resulted in a 'twin peak' quite distinct from previous seasons with longer pyrethroid windows (fig. 4). The apparent twin peak of 1986/87 was quite different from the 1989/90 season in that the trough occurred in the last two weeks of the Stage II pyrethroid window, not in Stage III (figs 4 and 11). This was the only season when pyrethroid resistance declined within the pyrethroid window and it coincided with a swing away from pyrethroids during the latter half of the Stage II window in this season (fig. 11), because of serious resistance problems.

Stage I levels returned to quite low levels (less than 10%) for the first three seasons but showed an alarmingly large increase early in the 1986/87 season (fig. 7). This coincided with high survival of the highly resistant overwintering pupae (44.5% average resistance for the previous Stage III, table 2) which were not destroyed by stubble cultivation during the 1986 winter because of record low prices on the New York cotton futures (table 6, fig. 12). In fact, the index of inter-season decline for this winter, was the lowest recorded during the study (table 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 6). When prices stabilized, stubble management and crop rotation practices also returned to normal (table 6). The following Stage I (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 three years of the strategy (fig. 7, table 2). In fact, overall, there has been a clear and steady increase in resistance levels, in all three Stages over time (fig. 7).

Pyrethroid resistance - Emerald

Resistance patterns were remarkably similar to those found in the Namoi/Gwydir area, but with some important differences. For example, the increases in resistance between Stages I and II, tended to be higher than in the Namoi/Gwydir, despite often lower selection pressure (table 3). This index of adult selection correlated well with the area of maize sown at Emerald, being highest when maize was a significant alternative crop in the irrigation area (table 3). There were also some differences

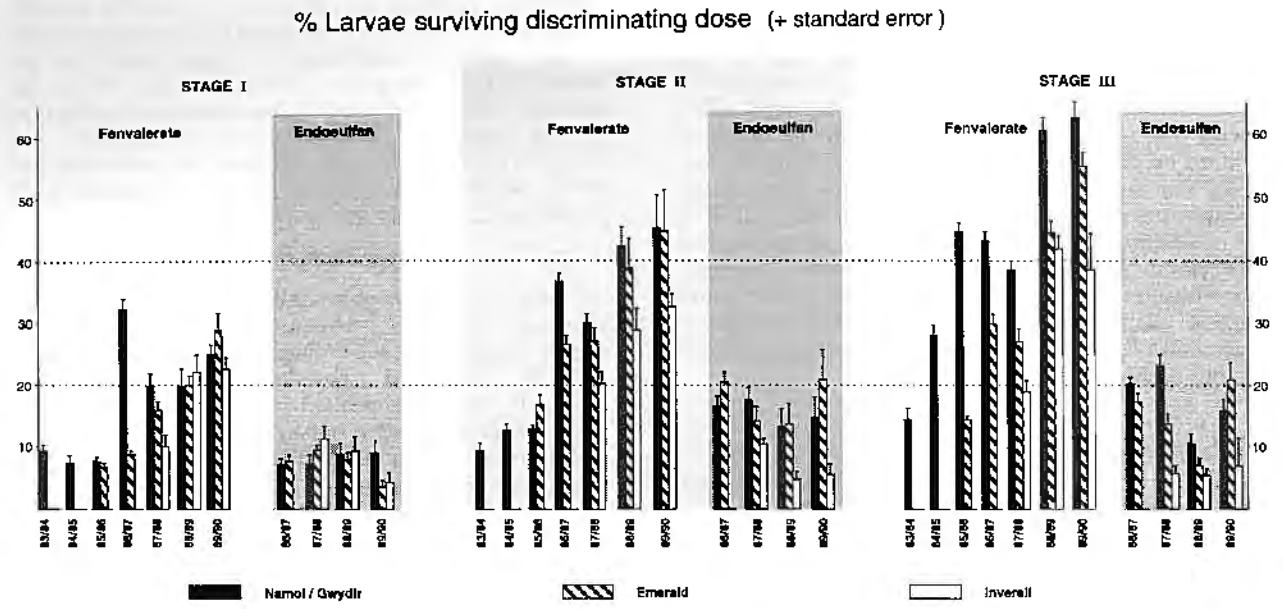


Fig. 7. Average pyrethroid and endosulfan resistance levels in *Helicoverpa armigera* for Stages I, II and III of the Resistance Management Strategy, for three study areas (the Namoi and Gwydir river valleys of northern New South Wales, the Emerald irrigation area of central Queensland and a sample of 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 μg of fenvalerate and endosulfan, respectively, per 30–40 mg larva) + the standard error of the mean.

Table 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:-

	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

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

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)

due to the longer season at Emerald, where Stages I and III each span multiple (two to three) generations, instead of a single generation as in the Namoi/Gwydir. This resulted in secondary lower resistance peaks late in Stage III (around April), about a generation after the first Stage III peaks in late February (fig. 5). The longer Stage III at Emerald also allowed more time for dilution, and Stage III resistance levels at Emerald were significantly lower than in the Namoi/Gwydir, despite similar Stage II levels (fig. 7, table 2). Despite these longer regression intervals (see Section 1) at Emerald, the trend towards steadily increasing resistance in all three Stages over time, noted in the Namoi/Gwydir, was also clearly evident at Emerald (fig. 7). Also, the Stage III 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. 5). The lack of a Stage III peak in 1985/86 was due

Table 2. Average pyrethroid and endosulfan resistance levels in *Helicoverpa armigera* for each Stage (I, II and 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 µg 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.

Study area	Season	Fenvalerate									Endosulfan								
		I			II			III			I			II			III		
		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

to the very low pyrethroid selection pressure in that season because of the late timing of the pyrethroid window (see Appendix 3).

The Stage I resistance levels for the first two seasons of monitoring at Emerald, were similar to the early Stage I figures for the Namoi/Gwydir (table 2). The 1985/86 Stage I 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). The 1987/88 Stage I data are also interesting as they clearly show a spring to early summer decline in pyrethroid resistance during the multiple generation Stage I period (fig. 5). However, recent Stage I patterns at Emerald have been quite variable and difficult to interpret (fig. 5).

The twin Stage II/Stage III peak noted for the 1989/90 shortened pyrethroid window season in the Namoi/Gwydir (fig. 4), was not so clearly evident at Emerald (fig. 5), probably because of lower pyrethroid use at this site.

Pyrethroid resistance - Inverell refugium

Pyrethroid resistance levels at the start of the season (Stage I) have increased to similar levels as those found in the nearby sprayed Namoi/Gwydir cotton area (fig. 7, table 2). The Stage II and III 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 refugium (fig. 7).

Dilution potential of refugia

The index of total season selection pressure (the increase in pyrethroid resistance between the start (Stage I) and the end (Stage III) of the season (table 5) is influenced by a complex interaction of factors selecting for and against resistance. Table 5 and figure 13 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. 13). 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, 1985/86, 1988/89), selection indices reflected closely the pyrethroid use in the sprayed cotton areas, without the confounding influence of immigration (table 5, fig. 13).

Endosulfan resistance - Namoi/Gwydir

Each season showed a similar pattern with the largest increases (up to 2.4-fold) between Stages I and II, and only smaller increases or none, between Stages II and III (table 2). These moderate mid/late season resistance levels always returned to low levels by the beginning of the following season (table 2, figs 6 and 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 Namoi/Gwydir but with some differences. The increases between Stages I and II were generally higher at Emerald (up to 6.8-fold, table 2) despite similar Stage I selection pressure (Appendix 3). The average Stage III figures at Emerald indicated little or no change between Stages II and III (table 2), whereas the weekly data indicated a clear response to Stage II selection pressure with sharp Stage III peaks (35-45%) in three out of four seasons (fig. 6). Similar high levels were only reached in one out of four seasons in the Namoi/Gwydir area (fig. 6). The longer Stage III season at Emerald would have masked these transient high levels when averaged over the entire Stage III period. As in the Namoi/Gwydir, these moderate to high mid/late season resistance levels always

Table 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 *Helicoverpa armigera*.

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
[b] data from figs 54 & 56
*, ^{ns} indicate Stage II Pyr levels are significantly higher ($P < 0.05$) or not significantly different, respectively, from Stage I levels (nnpaired t test)

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, figs 6 and 7).

Endosulfan resistance - Inverell refugium

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, fig. 7).

Resistance by host crop

During collecting trips, eggs were sampled from whatever hosts were available at the time. Obviously, few alternative hosts were available in the Namoi/Gwydir cotton monoculture but various crop hosts and weeds were available at Emerald and in the Inverell refugium. Table 7 indicates the periods when sufficient samples of more than one host could 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 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 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 (full bioassay of laboratory reared F_1 progeny) had been employed (see Section 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 (Boggild & Keiding, 1958; Roush & Miller, 1986; Dennehy, 1987). Field material can also be lost during laboratory culturing through prior parasitism (e.g. Suckling *et al.*, 1987), disease, escapes, rearing deformities and low copulation rates (e.g. Topper, 1987b) 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 parasitoids *Trichogramma* sp. and *Trichogrammatoidea* sp. (Hymenoptera: Trichogrammatidae) (especially at Emerald) and the egg/larval parasitoid *Chelonus* sp. (Hymenoptera: Braconidae) (mostly early season). 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 direct assay of field collected material (Martinson *et al.*, 1991), particularly so for moths (discussed more fully in Section 7). The technique also catered quite easily for the assay of field material from remote sites at a centralized testing laboratory, quite impossible with moth testing (Forrester, 1990a). It also allowed culling of the coincident sympatric species, *H. punctigera*, which is not possible with any of the techniques involving the screening of neonates reared from field collected eggs. These latter self dosing foliar

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

Winter	% Pyrethroid resistance (Pyr)[a]		Index of inter-season decline (decrease in Pyr between previous Stage III and the following Stage I)	% Cotton stubble[b] left uncultivated	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 and August
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
[b] data from table 6 (1986 data estimate only)
[c] data derived from fig. 12
** indicates Stage I Pyr levels are significantly lower ($P < 0.01$) than previous Stage III levels (unpaired t test)

residue tests are also subject to avoidance behaviour problems (Brown & Brogdon, 1987) and have, at least for *H. 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 *H. armigera* (Daly & Gregg, 1985; Farrow & Daly, 1987), 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 '*Helicoverpa* 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 (*Musca domestica* Linnaeus (Diptera: Muscidae)) (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, 1990a).

No attempt was made to convert the percentage resistance data (i.e. percentage 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 (e.g. Busch-Petersen & Wood, 1986). Given all these difficulties, it was decided not to attempt to convert the percentage 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. Firko (1991) and Daly & Fisk (1992) both advise similar caution when the mode of inheritance is unclear.

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 continually being 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 Section 7 and references therein). The slight loss in precision incurred by adopting the simpler pooled error estimate would be significant only for the pyrethroid screen component of the

Table 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 in *Helicoverpa armigera* from the Namoi and Gwydir river valleys of northern New South Wales.

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[c]	
	Stage I	Stage III			Summer rainfall[c] (% of long term mean)	Sorghum production[c] (% of six 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
[b] data from fig. 54
[c] data from fig. 10
** indicates Stage III Pyr levels are significantly higher ($P < 0.05$ and $P < 0.01$, respectively) than Stage I levels (unpaired t test)

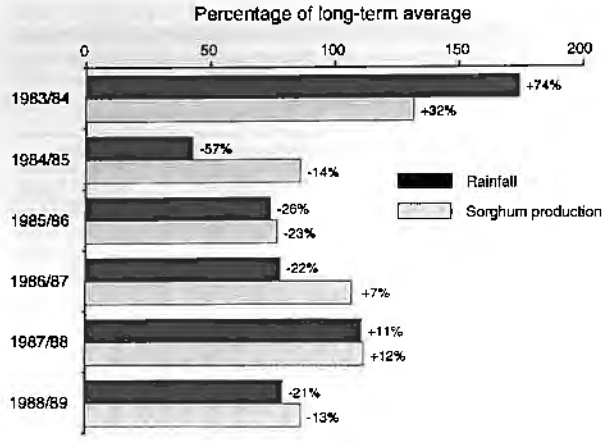


Fig. 8. Stage III dilution potential of the unsprayed raingrown cropping area of north-western NSW, surrounding the Namoi/Gwydir study site, as indicated by the impact of rainfall and its effect on the production of sorghum (the major Stage II flowering crop host for *Helicoverpa armigera* in this area). 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 (64.9 mm) 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 six year average (323,500 tonnes), data source Australian Bureau of Statistics and NSW Agriculture & Fisheries.

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 co-incident sibling species *H. punctigera*, especially in Stage I. All of the dicotyledonous hosts (e.g. cotton, sunflowers, soyabean) attract both species but the graminaceous hosts (sorghum and maize) attract only *H. 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 I period and especially in the Namoi/Gwydir cotton monoculture study area. This could then also introduce the possibility of further cost savings in laboratory 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, 1990a). Without this ability, growers, resellers, consultants and the agrochemical industry would probably not 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 II window, immediately following the first pyrethroid use; the other showing up in early Stage III, about a generation after the first pyrethroid use. The increase in early Stage III can be explained by selection of larvae, which would have developed through to egg laying moths by early Stage III. However, the immediate increase within the Stage II 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 Section 4), endosulfan selection on larvae in Stage I 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 Section 7) that nectar feeding adult Lepidoptera would not express metabolic resistance. However, it was quickly shown that *H. 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 Section 7). In fact, this adult selection was very sensitive to selection pressure as was seen in the 1986/87 Namoi/Gwydir Stage II 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 alternative crop to cotton. *H. armigera* oviposits on silking maize in late November/early December at Emerald, producing moths five to six weeks later, right at the start of the Stage II pyrethroid window. These moths emerge from the senescent maize blocks and fly directly 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 recognized 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 correlated very well with the area of maize grown, being highest when maize accounted for approximately a quarter of the cropping

Table 6. Impact of cotton price forecast (for the critical four month period after picking, May to August) on growers' stubble management and crop rotation decisions for the Namoi and 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,

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]					% 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 and August	
		a	b	c_i	c_{ii}	c_{iii}	Total	a	b_i	b_{ii}	b_{iii}	Total						
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 and 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) and (1a, 1b, 1ciii, 2biii), respectively

[6] Data derived from fig. 12

[7] Data from fig. 52

area and lowest when the maize area dropped to 10% or less.

While the Stage II window was 42 days, the Stage II adult selection peak ran on into the start of the larval selection peak, giving the impression of one sharp Stage III 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 III 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 II. The other exception was the 1989/90 season when the Stage II pyrethroid window was shortened to 35 days to avoid the double selection that was occurring in the sixth week (see Section 1). This achieved the desired impact as the two selection peaks were clearly separated, with the trough occurring in early Stage III. 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 I) and the end of the season (Stage III). This is better than using the Stage III 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 I spring/early summer levels were always significantly lower than the previous Stage III 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 *H. 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 & Cull (1984) also found high densities of overwintering pupae of *H. armigera* at Emerald (up to 30/m²) 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 III. Thus the overwintering population under cotton constitutes the major source for the carry over 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 recognized by Denholm *et al.* (1985) and Roush & McKenzie (1987). 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 Section 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 6) should only be undertaken in situations where overwintering pupal populations are low or absent. Cultivation

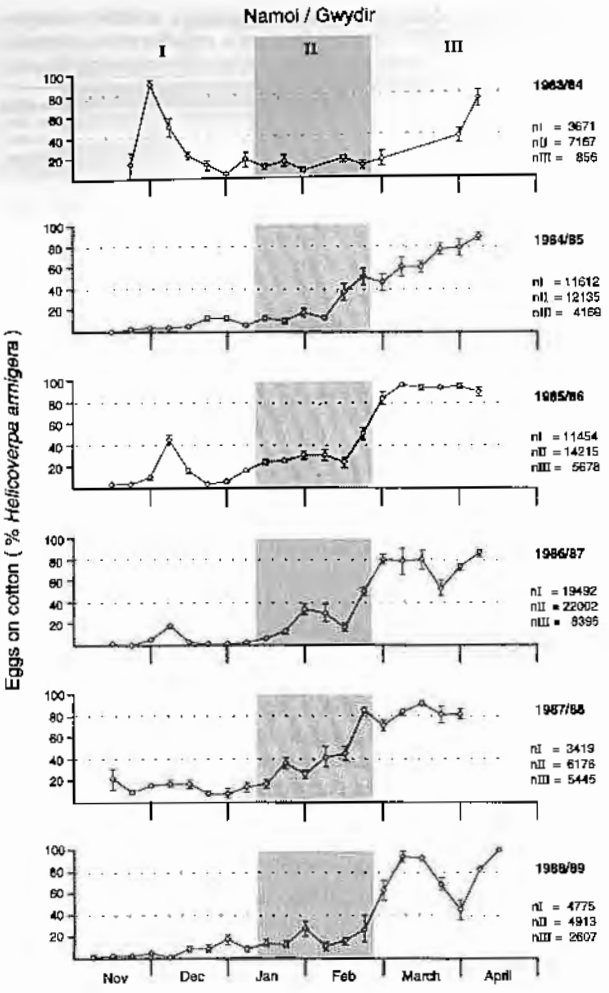


Fig. 9. Average weekly percentage *Helicoverpa armigera* (± standard error of mean) reared from eggs collected off cotton in the Namoi and Gwydir river valleys of northern NSW. nI, nII, nIII = the total number of larvae (*H. armigera* plus *H. punctigera*) reared for each Stage (I, II and III) of the six seasons since the introduction of the Resistance Management Strategy.

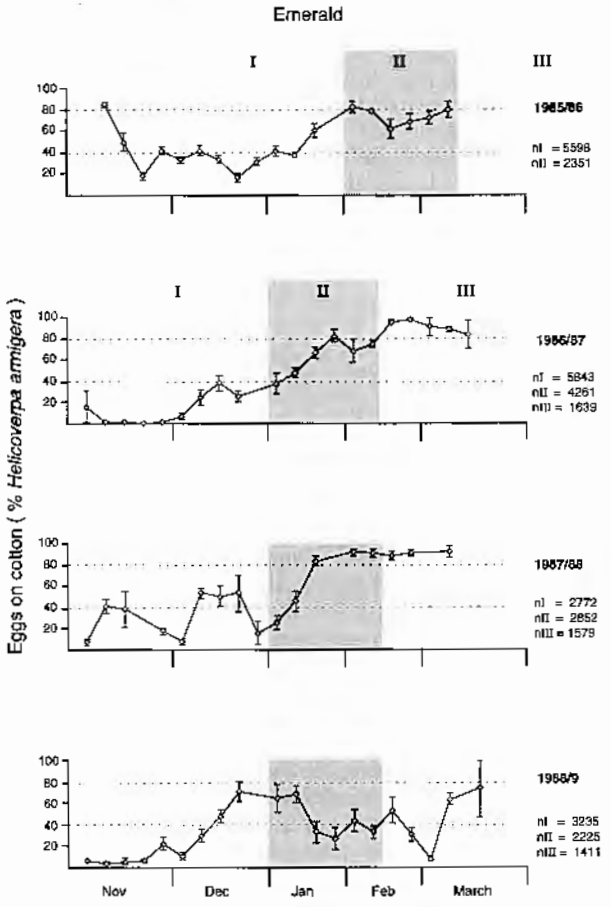


Fig. 10. Average weekly percentage *Helicoverpa armigera* (± standard error of mean) reared from eggs collected off cotton in the Emerald irrigation area of central Queensland. nI, nII, nIII = the total number of larvae (*H. armigera* plus *H. punctigera*) reared for each Stage (I, II and III) of the past four seasons of the Resistance Management Strategy.

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 *H. armigera* life cycle as it can remain vulnerable to simple 'resistance proof' physical control measures for almost six 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 recognized previously (Harris *et al.*, 1982; Macdonald *et al.*, 1983a; Hammock & Soderlund, 1986) and should be exploited more assiduously in the Australian strategy.

As mentioned previously, Stage I spring/early summer resistance levels were always lower than those in the preceding Stage III 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.*, 1988b). Commenting on a similar situation with *Heliothis virescens* (Fabricius) in the USA, Plapp *et al.* (1990b) suggested that reproductive disadvantage of resistant moths was the cause but ignored completely the equally plausible explanation of simple dilution. Stage I resistance levels in the early years of the strategy showed little variation even during the multiple generation Stage I periods at Emerald. However, as resistance levels have increased over time, so has the variation in Stage I 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 I 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

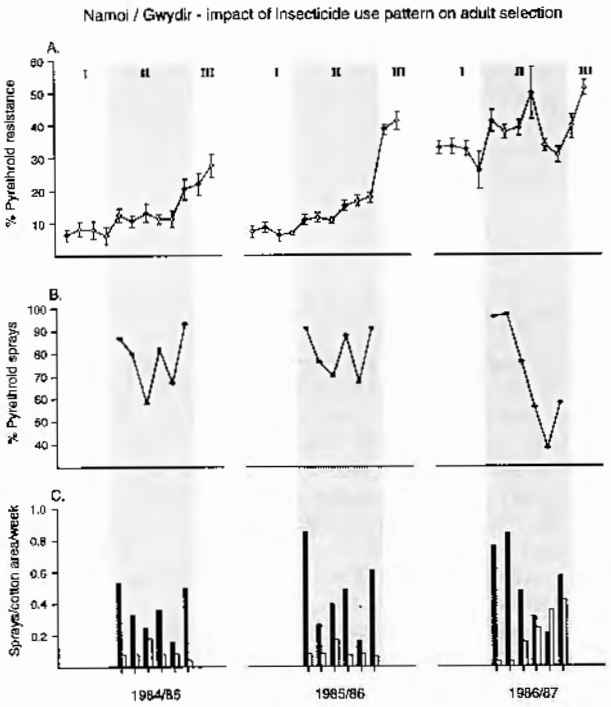


Fig. 11. A. Weekly pyrethroid resistance (% of *Helicoverpa armigera* larvae, reared from field collected eggs, surviving the fenvalerate discriminating dose ± between site binomial standard error) from the Namoi and Gwydir river valleys of northern NSW for the Stage II pyrethroid window, as well as the four weeks before and the two weeks after.

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

C. 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 six weeks of the Stage II pyrethroid window.

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 two to three 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 *Helicoverpa armigera* pressure and/or premating selection which occurs at Emerald.

The 1985/86 Stage I 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 (*Haematobia irritans* (Linnaeus) (Diptera: Muscidae)) in Illinois after two 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 refugium of susceptibles slows the evolution of resistance (Georghiou & Taylor, 1976,1986; Leeper *et al.*, 1986; National Research Council, 1986; Roush & Croft, 1986; Mason *et al.*, 1989). However, it has also been suggested that as the refugium 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 resis-

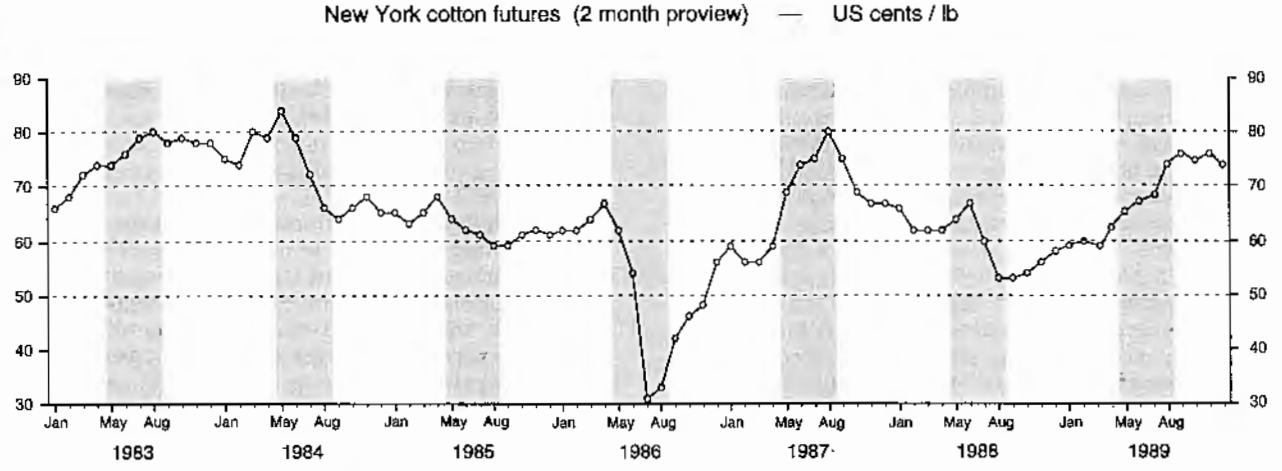


Fig. 12. Average monthly New York cotton futures price for the past seven years of the Resistance Management Strategy. Shaded areas represent the four month late autumn/winter period (May–August) when Australian cotton growers are making stubble management and crop rotation decisions after the end of picking in March/April. Data source, Namoi Cotton Co-operative, Wee Waa NSW and Merill Lynch Pty Ltd, Sydney.

tance 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* Wiedemann (Diptera: Culicidae) 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 has not overcome the problem. It has simply allowed more time (Hammock & Soderlund, 1986; Sawicki & Denholm, 1987) to allow the discovery, development and implementation of alternative control measures, both chemical and non-chemical (e.g. see work on synergists and resistance breaking pyrethroids in Sections 9 & 10).

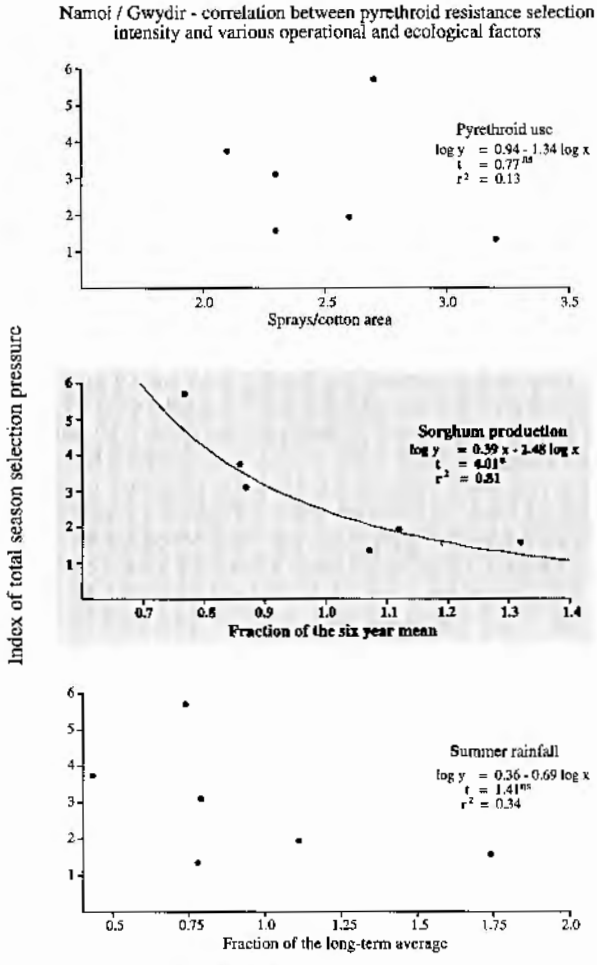


Fig. 13. Index of total season selection pressure for pyrethroid resistance in *Helicoverpa armigera* from the Namoi and Gwydir river valleys of northern NSW, as influenced by pyrethroid use, summer rainfall and sorghum production. Data (derived from table 5) for the six seasons since the introduction of the resistance Management Strategy. *, ns indicate significant (P <0.05) or non significant regression, respectively. (r^2 = coefficient of determination).

Endosulfan resistance

Endosulfan is one of the key insecticides for control of *Helicoverpa* spp. in Australian cotton. Endosulfan resistance in *H. armigera* has been a problem since the early 1970s (see figure 1 in Section 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 I, although a smaller amount is also used in Stage II (see Appendix 3). Therefore, larval selection should be manifested principally in the following generation in Stage II with a smaller response in the Stage III 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 I period, suggesting little impact of adult selection with endosulfan. The endosulfan responses at Emerald were generally higher than those in the Namoi/Gwydir, despite similar selection pressure. This can probably be attributed to the higher *H. armigera* pressure at Emerald, particularly during the period of intense endosulfan use in Stage I.

Endosulfan resistance in the unsprayed refugium 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 may promote effective dispersal of pyrethroid resistant moths (see Section 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 and 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 remain 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 I when *H. armigera* numbers are at their lowest. Therefore, actual selection pressure on *H. armigera* could well be lower for endosulfan than that

Table 7. Percentage pyrethroid and endosulfan resistance in *Helicoverpa 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).

Site	Year	Collection period		Host					
		Stage	Weeks	Cotton	Maize	Sunflowers	Soybean	Sorghum	*V. virgatum*
FENVALERATE									
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)			

for fewer pyrethroids directed at higher *H. armigera* populations in Stage II. 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 Section 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 and larvae) would result in more severe resistance problems than endosulfan selection on only one life stage (larvae).

A further possible explanation is a difference in the dominance in the expression of the major resistance genes. It is widely recognized that resistance is much more easily controlled if it is recessive (Curtis *et al.*, 1978; Wood & Bishop, 1981; Georghiou, 1983; Curtis, 1985; Croft & van de Baan, 1988). The major pyrethroid resistance mechanism in Australian *H. armigera* (metabolic detoxification) has been found to be controlled by a semi-dominant gene (Gunning & Easton, 1987; Daly, 1988; Daly & Fisk, 1992). However, the genetics of the endosulfan resistance gene remains unknown, although other cyclodiene resistance genes in various organisms have been shown to be intermediate in expression, ranging from incompletely recessive to incompletely dominant (Brown & Pal, 1971; Wood & Bishop, 1981; Oppenoorth, 1985; Busch-Petersen & Wood, 1986; Plapp, 1986; Bonner & Yarbrough, 1987). If a similar situation occurs with endosulfan resistance in *H. armigera*, then it is quite possible that the greater dominance in the expression 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 strategies or modifications to 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 were any increased use of endosulfan on the higher *H. armigera* populations which occur later on in the Stage II period. Up until now, endosulfan has had only a small but consistent use in Stage II (15-20% of Stage II 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 Stage II as an alternative to break up consecutive use of pyrethroids or as a clean-up spray for pyrethroid failures especially at Emerald where endosulfan now accounts for almost 40% of Stage II sprays (see Appendix 3). This increased use of endosulfan in Stage II 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 used increasingly in Stage II, 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 minimize the use of endosulfan in Stage II and the best way to do this is to preserve the efficacy of the pyrethroids (various options discussed in Sections 8, 9 and 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 along with a strong recommendation for the use of ovicidal mixtures (see Section 1). The rotation component of this strategy has proved 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 (e.g. Georghiou & Taylor, 1977; Comins, 1986; Via, 1986) as well as an increasing number of practical studies indicating the same (e.g. Macdonald *et al.*, 1983a, Flexner *et al.*, 1988; Immaraju *et al.*, 1990a). 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; Curtis, 1985, 1987; Mani, 1985; Comins, 1986; Leeper *et al.*, 1986; National Research Council, 1986; Holloway & McCaffery, 1988; Mallett, 1989; Roush, 1989) 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 (e.g. 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.

Section 3

Evaluation of the impact of the strategy on pyrethroid resistance: F1 analyses

Summary

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 the 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 insecticide 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 section 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 the survivors of the discriminating dose field screens rather than randomly collected field populations. This modification had been suggested as a possibly more effective source of information as far back as 1960 (Davidson, 1960) 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 and materials

Tolerance curves

The sampling procedure, areas and processing were as described in Section 2. The fenvalerate discriminating dose survivors for each Stage were reared through to adults, randomly mated and tested as 30-40 mg third or fourth 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 figures 15, 16, 17 and 18. 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 *Helicoverpa armigera* were also obtained (fig. 14). 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 figure 14 were superimposed on each tolerance curve in figures 15, 16, 17 and 18.

Fenvalerate was tested on Namoi/Gwydir material from the inception of the strategy in the 1983/84 season until Stage I in the 1986/87 season. Fenvalerate testing at Emerald did not begin until the third year of the strate-

gy (1985/86 season) and finished at the same time as at 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 15, 16 and 17). (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. 18).

Beeman-Nanis analysis

Beeman & Nanis (1986) 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* (Herbst) (Coleoptera: Tenebrionidae)). 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 (1986) 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 four Emerald field populations of *H. armigera*, to one generation of selection with a discriminating dose of fenvalerate (0.2 µg/30-40 mg larva), are given in figure 19. Each data point represents the overall average for each Stage of the resistance management strategy from Stage I, 1983/84 to Stage I, 1986/87 in the Namoi/Gwydir and Stage I, 1985/86 to Stage I, 1986/87 at Emerald. The parental generation data come from table 2 and the F1 generation data were derived from figures 15 and 16. The predicted response line in figure 19 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. 19) 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. 19). 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. 14). This has been also noted by Daly (1988) and Daly & Murray (1988). The heterozygote tolerance

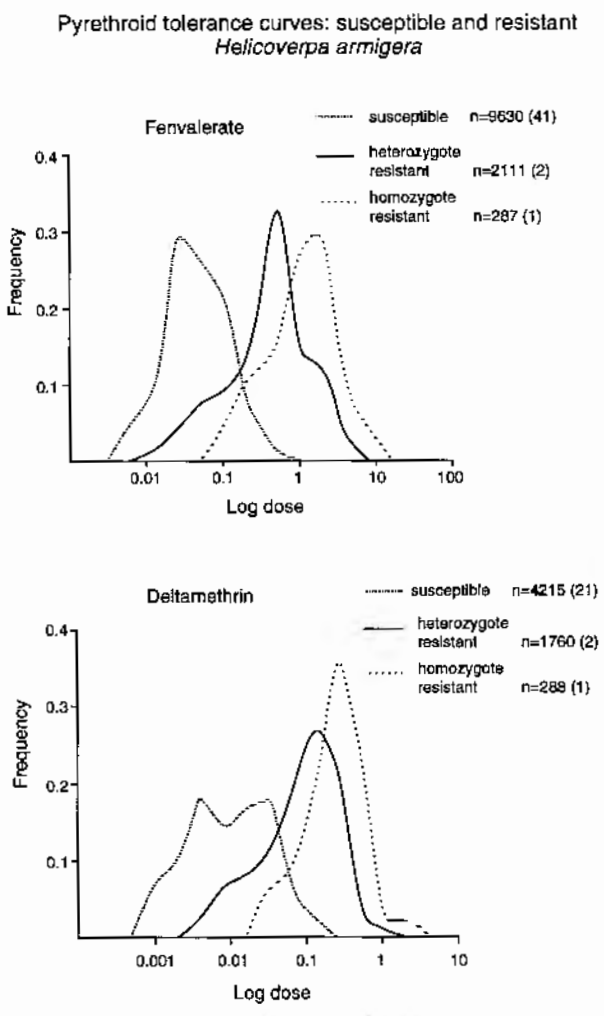


Fig. 14. Tolerance curves (after Via, 1986) for the fenvalerate and deltamethrin bioassay of susceptible and pyrethroid resistant *Helicoverpa armigera*. Abscissa, log dose (µg/30–40 mg larva). Ordinate, incremental kill frequency for that dose. Homozygote resistant colony pure breeding for a pyrethroid 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.

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 figure 14 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

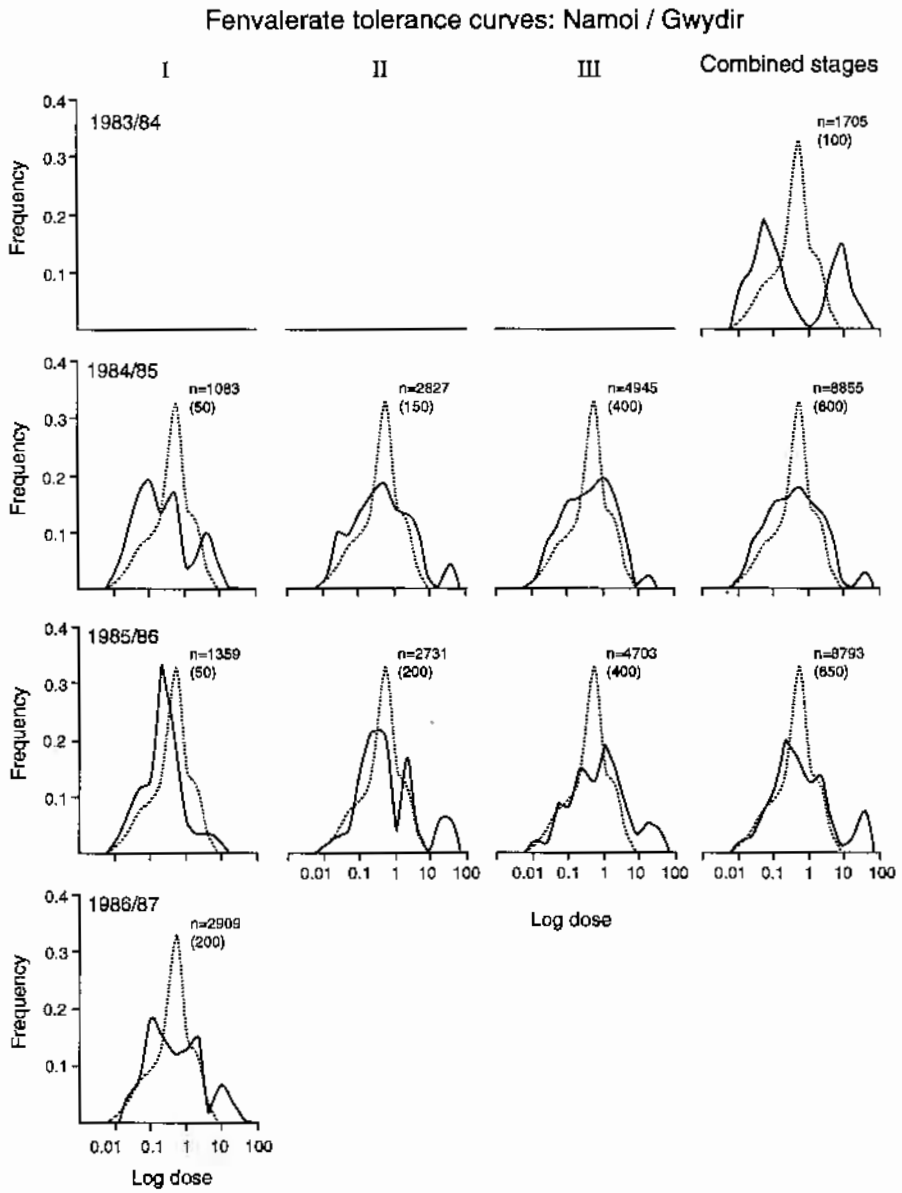


Fig. 15. Tolerance curves (after Via, 1986) for the fenvalerate bioassay of F1 progeny of *Helicoverpa armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir study area (1983/84 to 1986/87). Abscissa, log dose (µg/30–40 mg larva). Ordinate, incremental kill frequency for that dose. 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 14.

Namoi/Gwydir and Emerald (figs 15 and 16, 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 heterozygote tolerance curve. Peaks and plateaux to the left of the heterozygote tolerance curve (around 0.03 µg/larva, from fig. 14) indicate the presence of susceptibles, as would be expected in the F1. Peaks and plateaux to the right of the heterozygote tolerance curve (around 2.0 µg/larva, from fig. 14) 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 and 100 µg/larva), particularly in the Namoi/Gwydir 1983/84 season. In fact this particular season was anomalous in comparison to later years. There were two distinct population components 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 discus-

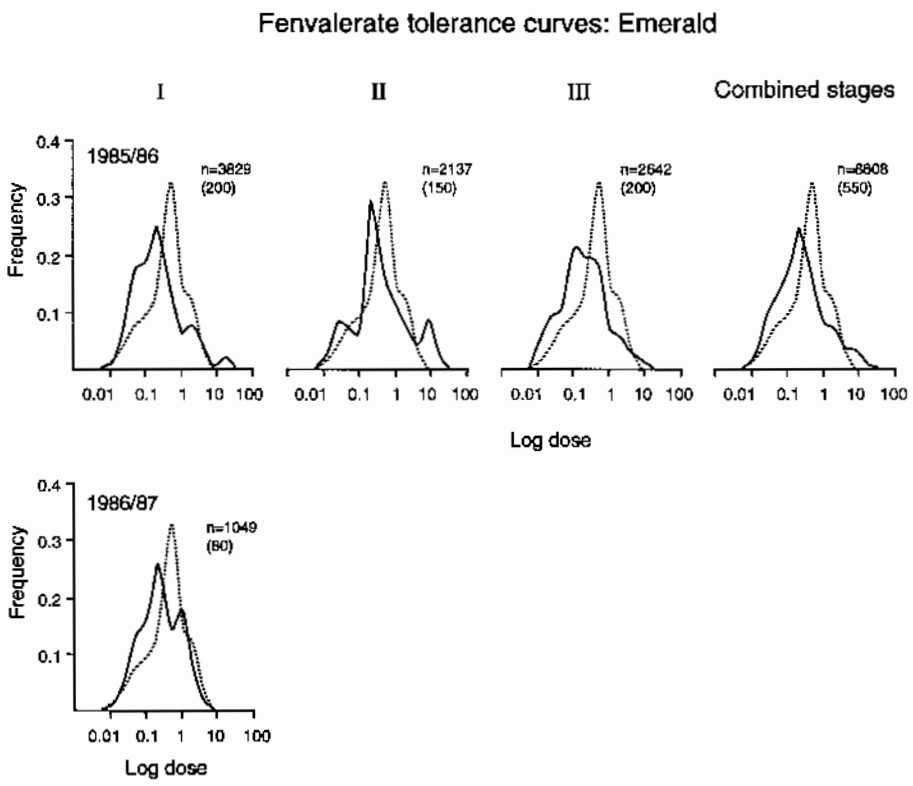


Fig. 16. Tolerance curves (after Via, 1986) for the fenvalerate bioassay of F1 progeny of *Helicoverpa armigera* fenvalerate discriminating dose survivors from the Emerald study area (1985/86 to 1986/87). Abscissa, log dose (µg/30–40 mg larva). Ordinate, incremental kill frequency for that dose. Combined = Stages I, II, III together. n = total number of larvae tested. Number of putative female parents in brackets. Dotted background fignre 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 14.

sion, that the Emerald site was not investigated until two years later, i.e. the 1985/86 season).

The deltamethrin tolerance curves in the Namoi/Gwydir and Emerald (fig. 17) indicate a similar situation to that 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 figure 14) could be detected quite distinctly but the homozygotes (at about 0.3 µg/larva, from figure 14) were more difficult to discern. As for fenvalerate, small highly resistant populations could be detected (between 0.5 and 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. 19), consistently and significantly overestimating the proportion of susceptibles in the F1.

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.* (1991) 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

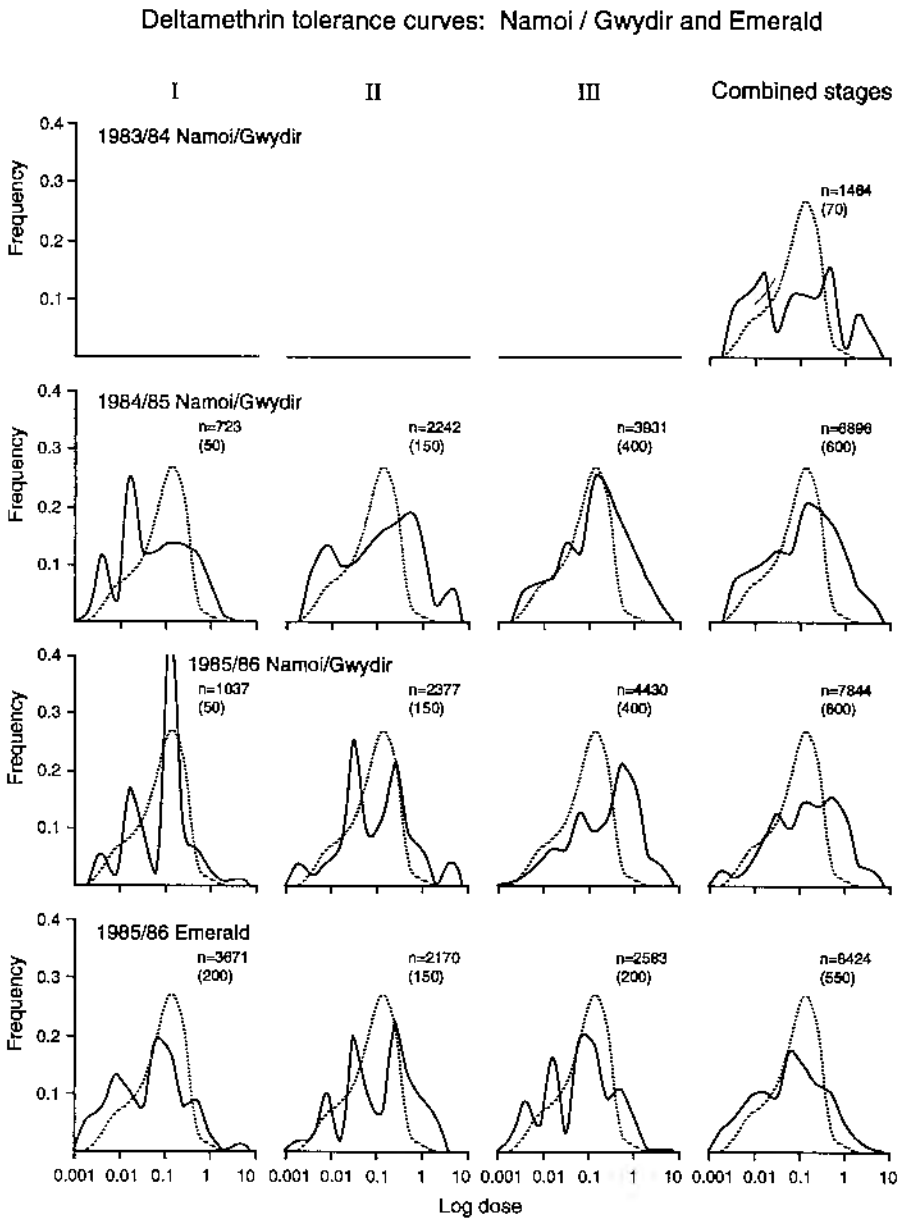


Fig. 17. Tolerance curves (after Via, 1986) for the deltamethrin bioassay of F1 progeny of *Helicoverpa armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir (1983/84 to 1985/86) and Emerald (1985/86) study areas. Abscissa, log dose (µg/30–40 mg larva). Ordinate, incremental kill 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 14.

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 Sections 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 suscepti-

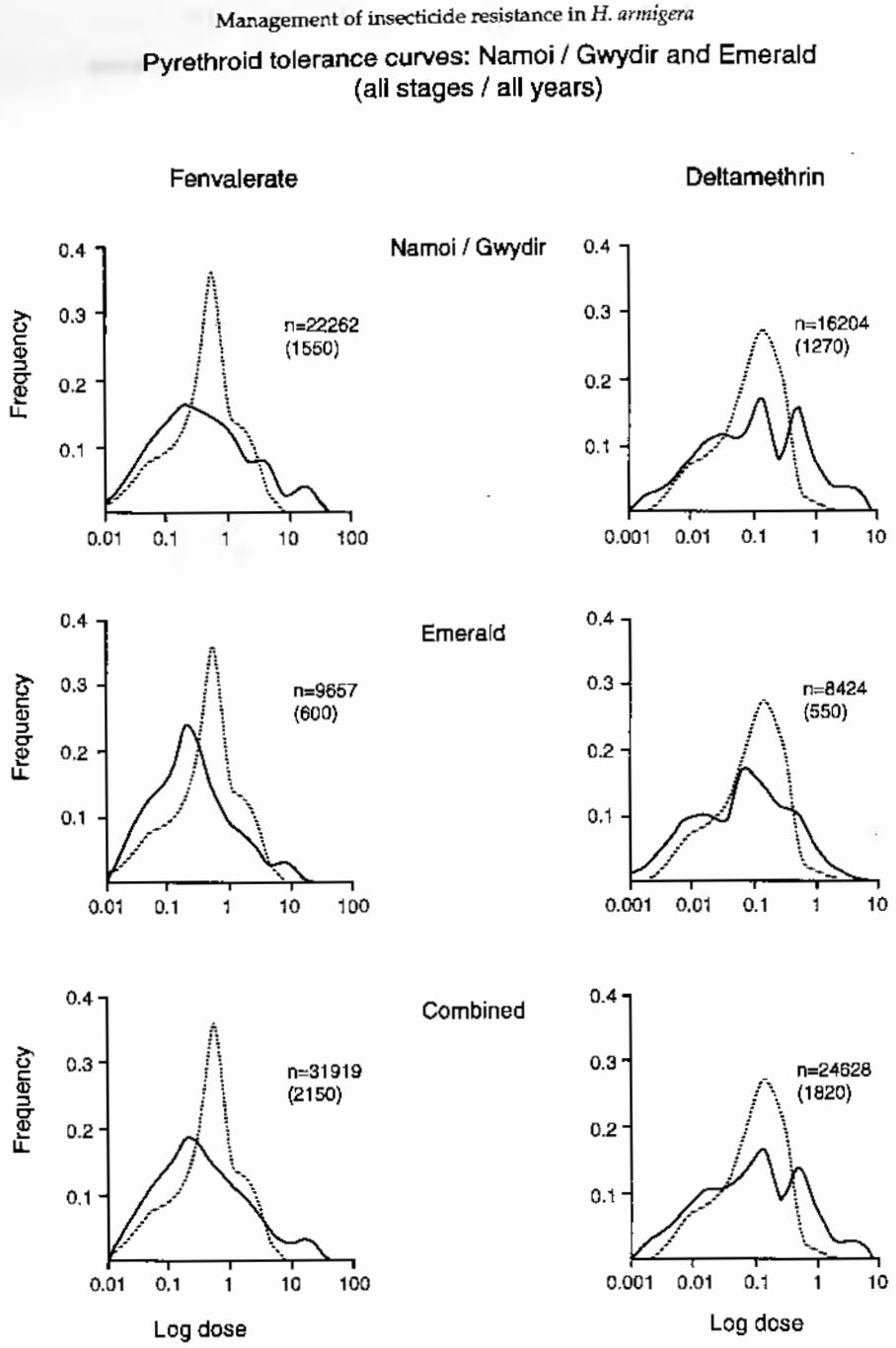


Fig. 18. Tolerance curves (after Via, 1986) for the fenvalerate and deltamethrin bioassays of F1 progeny of *Helicoverpa armigera* fenvalerate discriminating dose survivors from the Namoi/Gwydir and Emerald study areas, singly and combined. Data from all stages and all years (1983/84 to 1986/87) pooled. 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 suppresible by piperonyl butoxide (presumably a microsomal monooxygenase), from figure 14.

ble and heterozygote lines, will underestimate resistance and therefore overestimate susceptibility in the F1 generation. This 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 Section 5). The Beeman-Nanis analysis will probably only be of

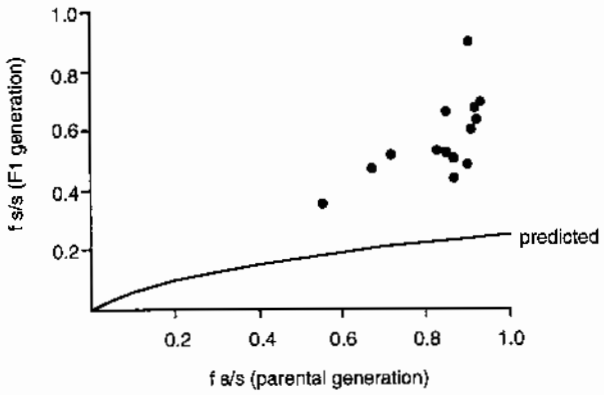


Fig. 19. Beeman-Nanis analysis of F1 data (see text for details).

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 is described fully in Section 8.

Section 4

Pyrethroid and endosulfan resistance: selection and cross resistance studies

Summary

Resistance to endosulfan and pyrethroids in *Helicoverpa armigera* in Australia was shown to be due to multiple rather than cross resistance. The independence of the endosulfan and pyrethroid resistance mechanisms vindicates the sequential use of these two groups in Stages I and II of the insecticide resistance management strategy, respectively. Within the cyclodienes, greatest resistance occurred to dieldrin with lower order cross resistance to endosulfan and endrin. Male and female moths expressed cyclodiene resistance equally.

Introduction

The basic principle underlying the Australian insecticide resistance management (IRM) strategy described in Section 1, is the rotation of unrelated chemical groups on a per generation basis. The success of such an alternation type strategy is dependent on the lack of cross resistance between sequential groups (Brown, 1982; Georghiou *et al.*, 1983; Tabashnik, 1989). In the Australian strategy, endosulfan is used intensively in Stage I , pyrethroids in Stage II and organophosphates/carbamates in Stage III (see Appendix 3). Stage I accounts for about half of all the insecticide used against *Helicoverpa* spp. on cotton in Australia (fig. 53), so it is essential that the intensive endosulfan selection in Stage I should not also select for pyrethroid resistance in the following generation.

A survey of the literature indicated little evidence for cross resistance between pyrethroids (or DDT) and any of the cyclodiene group of insecticides (table 8). Nor should this have been suspected as the principal modes of action of pyrethroids and cyclodienes are quite distinct. The pyrethroids act principally on the voltage sensitive sodium channel but also on secondary sites such as the Gaba receptor-chloride ionophore complex, the voltage sensitive calcium channel and the peripheral benzodiazepine receptor (Baillie, 1987; Ramadan *et al.*, 1988; Soderlund & Bloomquist, 1989; Soderlund *et al.*, 1989). The cyclodienes act by blocking Gaba-dependent chloride flux at the Gaba receptor-chloride ionophore complex but also act on the calcium regulatory mechanism in nerve terminals and cause excessive release of acetylcholine at synapses (Brooks & Mace, 1987; Ramadan *et al.*, 1988; Soderlund *et al.*, 1989). The Gaba receptor ionophore complex is a potential common site of action for pyrethroids and cyclodienes but it has been suggested that cyclodienes act at the picrotoxinin binding site on the Gaba receptor whereas pyrethroids interact with a closely associated yet distinct site (Lawrence & Casida, 1983; Casida & Lawrence, 1985; Ramadan *et al.*, 1988). Thus, theoretically at least , there should be no cross resistance between pyrethroids and cyclodienes. The history of cyclodiene resistance in Australia would also seem to support this. Resistance to the cyclodienes (endrin, endosulfan, toxaphene) first occurred in the early 1970s and remained high until the introduction of the pyrethroids in the late 1970s (figs 1 and 20). Endosulfan resistance levels then dropped dramatically, not recrudescing until the development of pyrethroid resistance and the subsequent implementation of the IRM strategy which necessarily promoted endosulfan use at the expense of pyrethroids. This retrieval of cyclodiene efficacy after the introduction of pyrethroids was also noted by Kay *et al.* (1983) and has also been noted for other pest/resistance situations (Burts & Croft, 1990; Plapp *et al.*, 1990a). Thus, practical experience and basic biochemical theory, would both seem to indicate little chance of cross resistance between pyrethroids and endosulfan. However, bearing in mind the previous, albeit few, positive records and the early reports of cross resistance in *Helicoverpa armigera* in Australia (table 8), it was decided to research this potential problem further.

Methods and materials

Selection studies

During the first three years of the Strategy, various colonies were formed from the survivors of the fenvalerate discriminating dose screens. There were three

Table 8. Literature survey for cross resistence records between pyrethroids or DDT and cyclodienes (dieldrin, endrin, aldrin, endosulfan).

Species	Recorded resistance to		Reference
	Pyrethroids	Cyclodienes	
Culex quinquefasciatus	✔	✖	Priester & Georghiou (1980)
Culex quinquefasciatus	✔	✖	Halliday & Georghiou (1985)
Helicoverpa armigera (India)	✔	✖	McCaffery *et al.* (1989a)
Helicoverpa armigera (Thailand)	✔	✖	Ahmad & McCaffery (1988)
Musca domestica	✔	✖	Scott & Georghiou (1986)
Gambusia (mosquito fish)	✖	✔	Bonner & Yarbrough (1987)
Helicoverpa armigera (Australia)	✔	✔	Gunning (1984), Twine (1984)
Earias vittella	✔	✔	Saini *et al.* (1989)
Spodoptera littoralis	✔	✔	Riskallah *et al.* (1983)
	DDT	Cyclodienes	
many insect Orders	✔	✖	Brown & Pal (1971)
many insect Orders	✖	✔	Brown & Pal (1971)
many Diptera/Coleoptera	✖	✔	Harris (1977)
Blattella germanica	✖	✔	Kadous *et al.* (1983)
Drosophila melanogaster	✔	✔	Brown & Pal (1971)
Aedes aegypti	✔	✔	Brown (1967)
Aedes taeniorhynchus	✔	✔	Brown (1967)

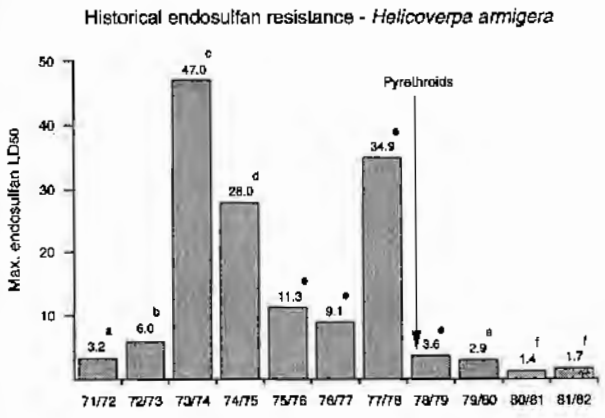


Fig. 20. Maximum endosulfan LD50s (µg/30–40 mg larva) recorded in the literature for *Helicoverpa armigera* larvae collected from the summer cropping areas of northern NSW and southern and central Queensland, for each season from 1971/72 (first available record) until the development of pyrethroid resistance in 1982/83. Letters refer to the following references; a Wilson (1974), b Goodyer *et al.* (1975), c Greenup (unpublished data), d Kay (1977), e Kay *et al.* (1983), f Gunning & Easton (unpublished data). Vertical arrow indicates the first season of significant use of synthetic pyrethroids. Average LD50 for susceptibles (0.75 µg/30–40 mg larva) from table 39.

colonies formed in 1983/84 season (one from the Namoi/Gwydir table 9, figure 21 and two from the Darling Downs area of southern Queensland, table 10, figure 22); one in 1984/85 season (Emerald, table 11, figure 23) and five in 1985/86 season (two from the Namoi/Gwydir, table 13, figure 25 and three from Emerald, tables 11 and 12, figures 23 and 24). These nine colonies were then subjected to either increasing pyrethroid or cyclodiene selection pressure in the laboratory (details given in tables 9, 10, 11, 12, 13, 14 and 15) and full bioassay lines were performed on subsequent generations.

The introduction of the endosulfan discriminating dose screen in 1986/87 season allowed ready access to endosulfan resistant field material. The pooled survivors from the 1986/87 Namoi/Gywdir and Emerald endosulfan screens were further selected with increasing endosulfan doses for seven generations after which selection pressure was switched to fenvalerate (table 14). The expression of cyclodiene resistance in adult *H. armigera* was followed over the seven generations of early endosulfan selection pressure on this colony (table 15). The bioassay techniques for larvae and adults (topical eye test) and statistical analysis methods are as indicated in Appendix 2. Resistance factors were expressed as LD50 selected strain ÷ LD50 of the susceptible strain (susceptible data from tables 37, 38 and 39). The bioassay data from tables 9, 10, 11, 12, 13, 14 and 15 were expressed graphically in figures 21, 22, 23, 24, 25, 26 and 27.

Cross resistance studies

Various populations of *H. armigera*, collected as eggs or larvae in the field, were bioassayed in the F1-3 with pyrethroids (deltamethrin or fenvalerate) or cyclodienes (dieldrin, endosulfan or endrin). Cross resistance relationships were determined by regressing the paired LD50s of colonies tested concurrently with various pyrethroids or cyclodienes (table 16). The data in this table include colonies collected from all cotton growing areas for all seasons from 1983/84 to 1987/88. The number of paired comparisons and regression equation components are given in table 16 for each possible cross resistance situation.

Results

Selection studies

Pyrethroid selection pressure resulted in elevated levels of pyrethroid resistance (up to >1000x, figure 23) but had no significant impact at all on cyclodiene resistance (figs 21, 22, 23 and 26). Cyclodiene selection pres-

Table 9. Impact of deltamethrin and endosnlfan selection on pyrethroid and endosnlfan resistance in larvae of a colony of *Helicoverpa armigera* established from survivors from the 1983/84 Namoi/Gwydir fenvalerate screens. Colony split three ways at F1 and selected with either deltamethrin or endosulfan or left nnselected. LD50 expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD50 resistant strain ÷ LD50 susceptible strain. Susceptible strain LD50s 0.01, 0.03 and 0.7 for deltamethrin, fenvalerate and endosulfan, respectively (data from tables 37, 38 and 39).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Deltamethrin				Fenvalerate				Endosulfan			
		LD50 (95% Confidence limits)		Slope	RF	LD50 (95% Confidence limits)		Slope	RF	LD50 (95% Confidence limits)		Slope	RF
Field													
F1	fenvalerate 0.2	0.24	(0.179, 0.321)	1.6	23.8	0.50	(0.349, 0.76)	1.2	16.7	4.1	(2.76, 6.14)	1.3	5.8
F2	deltamethrin ≥0.5	0.21	(0.152, 0.296)	1.3	20.9					0.9	(0.64, 1.18)	2.0	1.3
F3	deltamethrin ≥0.5	0.36	(0.119, 0.842)	0.5	35.6	0.67	(0.343, 1.27)	0.7	22.3	1.1	(0.61, 1.54)	1.5	1.5
F2	endosulfan ≥5	0.05	(0.026, 0.07)	1.6	4.8	0.41	(0.278, 0.59)	1.3	13.7	1.9	(1.43, 2.53)	2.0	2.7
F2	nil	0.14	(0.095, 0.209)	1.4	13.6	0.41	(0.274, 0.663)	1.0	13.7	1.7	(1.29, 2.19)	2.4	2.4

Table 10. Impact of fenvalerate and endosulfan selection on pyrethroid and endosulfan resistance in larvae of two colonies of *Helicoverpa armigera* established from fenvalerate discriminating dose survivors from the Darling Downs area of southern Queensland in 1983/84. Colony 1 split at F1 and selected with either fenvalerate of endosnlfan. LD50 expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD50 resistant strain ÷ LD50 snsceptible strain. Susceptible strain LD50s 0.03 and 0.7 for fenvalerate and endosulfan, respectively (data from tables 37 and 39).

Generation number	Selection pressnre on previons generation (µg/30–40 mg larva)	Fenvalerate			Endosulfan		
		LD50 (95% Confidence limits)	Slope	RF	LD50 (95% Confidence limits)	Slope	RF
Colony 1							
Field							
F1	fenvalerate 0.2	0.07 (0.046, 0.112)	1.1	2.3	0.8 (0.27, 1.29)	1.0	1.1
F2	fenvalerate 0.2	0.24 (0.143, 0.349)	1.4	8.0	1.4 (0.87, 1.96)	1.5	2.0
F2	endosnlfan ≥5	0.07 (0.055, 0.098)	2.0	2.3	1.5 (1.07, 2.15)	1.5	2.2
Colony 2							
Field							
F1	fenvalerate 0.2	0.09 (0.072, 0.117)	1.6	3.1	4.4 (3.28, 5.70)	1.9	6.2
F2	fenvalerate 0.2	0.12 (0.056, 0.203)	0.8	3.9	2.8 (1.29, 29.3)	1.2	4.0
F3	fenvalerate ≥0.2	0.41 (0.276, 0.602)	1.2	13.6	0.9 (0.67, 1.13)	1.9	1.3

Table 11. Impact of fenvalerate selection on pyrethroid and cyclodiene resistance in larvae of two colonies of *Helicoverpa armigera* established from survivors from the Emerald fenvalerate screens in 1984/85 and 1985/86. LD50 expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD50 resistant strain ÷ LD50 susceptible strain. Susceptible strain LD50s 0.03, 0.7 and 2.9 for fenvalerate, endosulfan and dieldrin, respectively (data from tables 37 and 39).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Fenvalerate				Endosulfan				Dieldrin			
		LD50 (95% Confidence limits)		Slope	RF	LD50 (95% Confidence limits)		Slope	RF	LD50 (95% Confidence limits)		Slope	RF
1984/85													
Field													
F1	fenvalerate 0.2												
F2	fenvalerate 0.2												
F3	fenvalerate 0.2	0.52	(0.383, 0.739)	1.8	17.3								
F4	fenvalerate 1.0	2.09	(1.514, 2.894)	1.2	70	2.3	(1.76, 3.08)	1.9	3.3	2.9	(2.21, 3.68)	1.8	1.0
F5	fenvalerate 8.0	7.67	(6.252, 8.946)	1.4	256	3.1	(2.19, 4.20)	1.5	4.5				
F6	fenvalerate 16.0	9.13	(7.076, 11.969)	0.9	304	3.5	(2.71, 4.48)	1.9	5.0	5.1	(3.57, 6.76)	1.5	1.7
1985/86													
Field													
F1	fenvalerate 0.2	0.18	(0.151, 0.222)	1.5	6	1.9	(1.36, 2.68)	1.9	2.7	4.4	(2.87, 6.06)	1.5	1.5
F2	fenvalerate 2.0												
F3	fenvalerate 2.0												
F4	fenvalerate 2.0												
F5	fenvalerate 2.0	4.58	(3.274, 6.370)	0.9	153								
F6	fenvalerate ≥2.0	30.8	(19.39, 57.25)	0.7	1,026	3.7	(3.02, 4.61)	2.2	5.3	10.4	(7.16, 14.71)	1.1	3.6
F7	fenvalerate ≥16.0	86.2	(34.68, 792.1)	0.8	2,873)								

Table 12. Impact of cyclodiene selections on pyrethroid and cyclodiene resistance in larvae of two colonies of *Helicoverpa armigera* established from snrvivors from the 1985/86 Emerald fenvalerate screens. Colony 2 split at F1 and selected with either dieldrin or endosulfan. LD_{50} expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD_{50} resistant strain ÷ LD_{50} susceptible strain. Susceptible strain LD_{50}s 0.03, 0.7 and 2.9 for fenvalerate, endosulfan and dieldrin, respectively (data from tables 37 and 39).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Fenvalerate				Endosulfan				Dieldrin			
		LD_{50} (95% Confidence limits)		Slope	RF	LD_{50} (95% Confidence limits)		Slope	RF	LD_{50} (95% Confidence limits)		Slope	RF
Colony 1													
Field													
F1	fenvalerate 0.2	0.08	(0.055, 0.110)	1.3	2.7	5.2	(3.65, 7.30)	1.1	7.5	10.0	(5.62, 17.68)	0.7	3.5
F2	dieldrin 25.6	0.12	(0.089, 0.155)	1.5	3.9	12.3	(7.07, 18.12)	1.2	17.6	132.2	(83.5, 314.1)	1.2	46
F3	dieldrin 25.6	0.04	(0.031, 0.053)	2.0	1.4	21.6	(15.84, 28.71)	1.6	30.9	604.5	(200.8, 53,141)	0.6	208
Colony 2													
Field													
F1	fenvalerate 0.2	0.39	(0.279, 0.542)	1.2	12.9	3.1	(2.45, 3.83)	1.5	4.4	6.5	(4.76, 8.70)	1.2	2.2
F2	dieldrin 25.6												
F3	dieldrin 25.6												
F4	dieldrin 25.6	0.08	(0.055, 0.102)	1.4	2.5	13.0	(10.29, 16.21)	2.1	18.5	33.2	(26.70, 43.17)	1.0	11.5
F5	dieldrin 25.6					26.7	(19.95, 38.46)	1.4	38				
F2	endosulfan 10												
F3	endosulfan 10												
F4	endosulfan 10	0.14	(0.099, 0.188)	1.1	4.5	5.2	(4.15, 6.54)	1.7	7.5	13.1	(10.02, 16.64)	1.6	4.5

Table 13. Impact of cyclodiene selection on pyrethroid and cyclodiene resistance in larvae of two colonies of *Helicoverpa armigera* established from survivors from the 1985/86 Namoi/Gwydir fenvalerate screens. LD_{50} expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD_{50} resistant strain ÷ LD_{50} susceptible strain. Susceptible strain LD_{50}s 0.03, 0.7, 2.9 and 0.6 for fenvalerate, endosulfan, dieldrin and endrin, respectively (data from tables 37 and 39).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Fenvalerate				Endosulfan				Dieldrin				Endrin			
		LD_{50} (95% Confidence limits)		Slope	RF	LD_{50} (95% Confidence limits)		Slope	RF	LD_{50} (95% Confidence limits)		Slope	RF	LD_{50} (95% Confidence limits)		Slope	RF
Colony 1																	
Field																	
F1	fenvalerate 0.2	0.45	(0.405, 0.492)	1.2	14.9	1.6	(1.48, 1.72)	1.6	2.2	3.1	(2.79, 3.44)	1.7	1.1	2.1	(1.09, 3.58)	1.1	3.5
F2	dieldrin 25.6																
F3	dieldrin 25.6					–											
F4	dieldrin 25.6	0.31	(0.243, 0.404)	2.2	10.4	8.6	(6.77, 10.97)	2.1	12.2	164.5	(83.1, 975.5)	0.7	57				
F5	dieldrin ≥25.6	0.43	(0.335, 0.545)	2.0	14.3	30.8	(24.35, 38.61)	2.0	44.0	1,052.3	(474.1, 10,526)	1.3	363				
F6	dieldrin ≥102.4	0.53	(0.415, 0.658)	2.4	17.7	39.3	(30.89, 49.96)	2.2	56.0	715.7	(432.0, 9,079)	2.1	247	20.2	(13.85, 27.47)	1.6	34.0
Colony 2																	
Field																	
F1	fenvalerate 0.2	0.75	(0.564, 1.012)	1.0	25.0	2.8	(2.19, 3.50)	1.5	4.0	3.1	(2.79, 3.44)	1.7	1.1				
F2	endosulfan 10																
F3	endosulfan 10																
F4	endosulfan 10	0.71	(0.568, 0.911)	1.4	24.0	2.2	(1.80, 2.75)	1.6	3.2	3.5	(2.84, 4.23)	2.0	1.2				

sure (either dieldrin or endosulfan) resulted in elevated resistance to all cyclodienes (up to >1000x, 628x and 72x for dieldrin, endosulfan and endrin respectively, figure 26) but had no significant impact at all on pyrethroid resistance (figs 21, 22, 24, 25 and 26). Resistance factors were generally greatest for dieldrin with lower cross resistance to endosulfan and endrin. The most compelling example was the pooled 1986/87 population (fig. 26) which demonstrated an inability to increase its pyrethroid resistance level when challenged by endosulfan but clearly demonstrated its capability to do so when challenged later with fenvalerate. The dramatic decline in endosulfan resistance in this colony from $F_{8\text{-}11}$ under fenvalerate selection pressure should not necessarily be taken as an indication of negatively correlated resistance, as it could simply indicate the response after relaxation of selection pressure. A concurrent nil selection treatment after F8 would have helped to resolve this question. However, this was not the aim of this study, so the problem remains unaddressed at this stage.

Both female and male moths expressed resistance to both dieldrin and endosulfan (fig. 27). As for larvae, resistance factors were generally greater for dieldrin with lower cross resistance to endosulfan. Female moths had consistently higher resistance factors than males for endosulfan but male and female moths were equally resistant for dieldrin (table 15).

Table 14. Impact of endosulfan selection on pyrethroid and cyclodiene resistance in larvae of a colony of *Helicoverpa armigera* established from pooled survivors from the 1986/87 Namoi/Gwydir and Emerald endosulfan screens. Selection switched to fenvalerate after the eighth generation. LD50 expressed in µg/30–40 mg larva. Resistance factors (RF) expressed as LD50 resistance strain ÷ LD50 susceptible strain. Susceptible strain LD50s 0.03, 0.7, 2.9 and 0.6 for fenvalerate, endosulfan, dieldrin and endrin, respectively (data from tables 37 and 39).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Fenvalerate			Endosulfan			Dieldrin			Endrin		
		LD50 (95% Confidence limits)	Slope	RF	LD50 (95% Confidence limits)	Slope	RF	LD50 (95% Confidence limits)	Slope	RF	LD50 (95% Confidence limits)	Slope	RF
Field													
F1	endosulfan 10												
F2	endosulfan 10												
F3	endosulfan 10												
F4	endosulfan 10	0.33 (0.264, 0.403)	1.9	10.8	17.5 (13.95, 22.03)	1.4	24.9	77.9 (53.9, 121.8)	0.9	26.9	13.2 (10.66, 16.56)	1.7	22.0
F5	endosulfan ≥40	0.26 (0.204, 0.327)	1.4	8.6	31.4 (25.09, 40.10)	1.4	44.8	245.4 (147.5, 527.6)	0.7	84.6	15.6 (12.43, 19.75)	1.3	25.9
F6	endolsulfan ≥80	0.30 (0.237, 0.389)	1.3	10.1	53.2 (43.39, 66.56)	1.6	76.0	419.9 (247.4, 1,002.7)	0.8	145	27.6 (22.07, 35.56)	1.5	46.0
F7	endosulfan ≥160	0.25 (0.186, 0.335)	1.0	8.4	149.0 (109.7, 219.4)	1.1	213	>1,000			30.9 (24.60, 40.18)	1.5	51.5
F8	endosulfan ≥320	0.30 (0.234, 0.373)	1.5	9.9	439.8 (228.9, 1,332.9)	0.8	628	>1,000			43.1 (33.0, 60.23)	1.5	72
F9	fenvalerate ≥1.0	0.21 (0.155, 0.275)	1.8	6.9	110.6 (64.25, 258.9)	0.8	158						
F10	fenvalerate ≥1.0	1.76 (1.239, 2.549)	0.9	58.7	17.3 (7.43, 65.66)	0.7	24.7						
F11	fenvalerate ≥2.0	15.56 (8.375, 88.59)	1.1	519	4.7 (2.86, 6.71)	1.1	6.7	9.4 (3.90, 16.37)	0.7	3.3	3.0 (1.81, 4.29)	1.1	5.0

Table 15. Impact of endosulfan selection on cyclodiene resistance in adults of a colony of *Helicoverpa armigera* established from the pooled survivors from the 1986/87 Namoi/Gwydir and Emerald endosulfan screens. LD50 expressed in µg/200mg staudard 1 day old fed moth. Resistance factors (RF) expressed as LD50 resistant strain ÷ LD50 susceptible strain. Susceptible strain LD50s 0.52, 1.24, 5.62 and 8.61 for endosulfan female/male and dieldrin female/male, respectively (data from table 44).

Generation number	Selection pressure on previous generation (µg/30–40 mg larva)	Sex	Endosulfan			Dieldrin		
			LD50 (95% Confidence limits)	Slope	RF	LD50 (95% Confidence limits)	Slope	RF
Field								
F1	endosulfan 10							
F2	endosulfan 10							
F3	endosulfan 10							
F4	endosulfan 10	♀	11.0 (7.91, 15.38)	1.2	21.2	31.4 (4.51, 516.3)	0.6	5.6
		♂	7.1 (4.64, 10.49)	1.0	5.7	42.4 (18.1, 91.8)	0.8	4.9
F5	endosulfan ≥40	♀	12.9 (8.80, 18.34)	1.2	24.8	549.1 (429.8, 821.0)	2.9	97.7
		♂	14.3 (9.21, 21.71)	1.0	11.5	885.5 (537.1, 6, 173)	1.4	102.8
F6	endosulfan ≥80	♀	32.4 (18.48, 51.15)	1.2	62.3	>1,000		
		♂	42.3 (25.41, 69.94)	1.2	34.1	>1,000		
F7	endosulfan ≥160							
F8	endosnlfan ≥320	♀	35.3 (20.82, 57.81)	1.3	67.9	>1,000		
		♂	49.3 (35.55, 70.75)	1.8	39.8	>1,000		

Cross resistance studies

As expected, there was good correlation between the cyclodienes but none at all between the pyrethroids and any of the cyclodienes (table 16). The low slopes between dieldrin (independent variable) and endosulfan/endrin (dependent variables) once again indicate the low order cross resistance from dieldrin to endosulfan and endrin.

Discussion

On no occasion did pyrethroid or cyclodiene selection pressure result in a reciprocal increase in resistance. In addition, no correlation could be found between pyrethroid and cyclodiene resistance in paired bioassays. Thus, there is overwhelming evidence to conclude that pyrethroid and endosulfan resistance in *H. armigera* in Australia is due to multiple rather than cross resis-

Table 16. Cross resistance patterns (regression of paired LD_{50}s) for various cyclodiene and pyrethroid insecticides tested concurrently on a number of *Helicoverpa armigera* populations collected as eggs or larvae in the field and bioassayed in the F_{1-3} (data includes samples from all monitoring areas for all seasons from 1983/84 to 1987/88). n = number of LD_{50} (µg/30–40 mg larva) comparisons. r^2 = coefficient of determination. t values either very highly significant *** (P <0.001), significant* (P<0.05) or non significant ns (P <0.05).

Variable		n	Components of regression equation y = a+ b x			
Independent	Dependent		a	b	t	r^2
Between cyclodienes						
dieldrin	endosulfan	67	4.49	+0.03	19.1***	0.85
dieldrin	endrin	8	7.03	+0.04	2.99*	0.60
endosulfan	endrin	11	17.13	+0.07	2.65*	0.44
Between pyrethroids & cyclodienes						
dieldrin	fenvalerate	67	0.92	−0.0001	−0.25ns	0.00
dieldrin	deltamethrin	45	0.09	−0.01	−1.24ns	0.03
endosulfan	fenvalerate	154	0.68	−0.0001	−0.09ns	0.00
endosulfan	deltamethrin	111	0.05	+0.02	1.20ns	0.01
endrin	fenvalerate	11	0.74	−0.01	−1.38ns	0.18

tance and that the two resistances are regulated by independent resistance mechanisms. Thus, as mentioned in Section 2, the increases in pyrethroid resistance measured within the Stage II window, cannot be attributed to cross resistance from endosulfan selection in the previous generation. The cyclodiene resistance patterns found in this study (greatest resistance to dieldrin, lower cross resistance to endosulfan and endrin) agree well with the published literature (Yadav *et al.*, 1965; Brown & Pal, 1971; Busvine & Feroz, 1971; Oppenoorth, 1985). The ability of moths to express cyclodiene resistance was not surprising as Hamilton (1966) and Niemczyk & Lawrence (1973) found that cyclodiene resistance could be expressed in both larval and adult Coleoptera.

The trend to the increased use of endosulfan in Stage II to replace the decreasingly reliable pyrethroids (see Appendix 3) raises some interesting parallels to the problem discussed in this section: in particular, the question whether endosulfan use in Stage II can select for cross resistance to organophosphates which are used extensively in the following Stage III period. The mechanism of cyclodiene resistance has been mostly attributed to a less sensitive target site on the Gaba receptor ionophore complex (Kadous *et al.*, 1983; Matsumura, 1983; Oppenoorth, 1985; Bonner & Yarbrough, 1987; Soderlund *et al.*, 1989). However, glutathione S-transferases have also been implicated for lindane and endosulfan resistance (Ishida & Dahm, 1965 and Kern *et al.*, 1990, respectively). The glutathione S-transferases are of course an acknowledged resistance mechanism for many organophosphate insecticides (Dautermann, 1985), so it is interesting to speculate whether increasing endosulfan use in Stage II will present a problem for subsequent organophosphate use in the following generation in Stage III. There are already increasing reports of deteriorating efficacy of organophosphates in Stage III which are closely linked to high endosulfan resistance situations. There are also published reports of cross resistance between organophosphates and cyclodienes in a number of insect species (Alava & Lagunes Tejeda, 1976 cited in Brewer & Trumble, 1989; Bauernfeind & Chapman, 1985; Hoy & Cave, 1989). So there is clearly a pressing need to initiate an endosulfan/organophosphate selection and cross resistance study, similar to the endosulfan/pyrethroid study reported in this paper. This work has been initiated and results will be reported elsewhere.

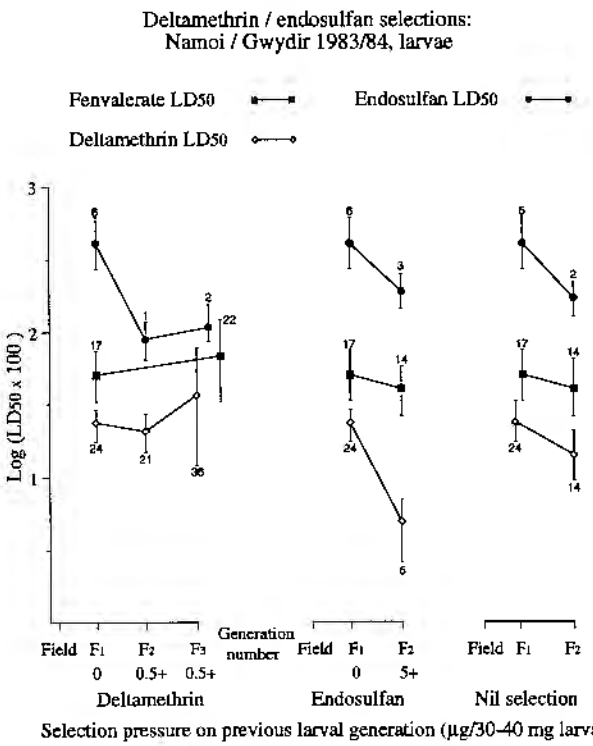


Fig. 21. Impact of deltamethrin, endosulfan and nil selection on pyrethroid and endosulfan resistance in larvae of a colony of *Helicoverpa armigera* established from survivors from the 1983/84 Namoi/Gwydir fenvalerate screens. Colony split three ways at F_1. Ordinate, log (LD_{50} × 100) (µg/30–40 mg larva) ± 95% confidence interval. Resistance factors above or below each confidence limit. Data from table 9.

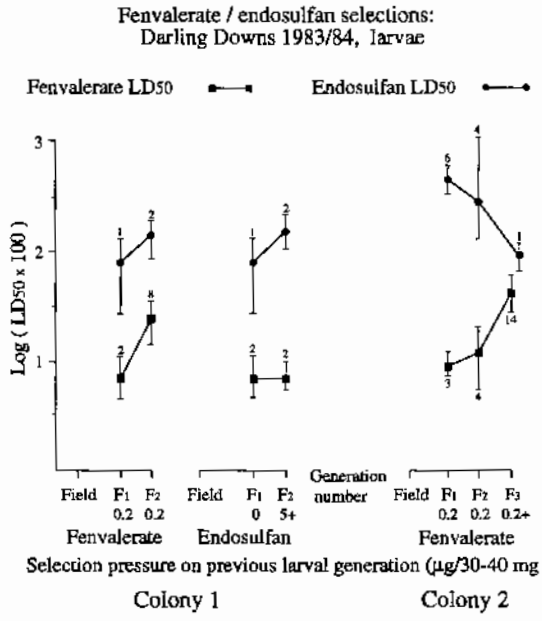


Fig. 22. Impact of larval fenvalerate and endosnlfan selection on resistance in larvae of two colonies of *Helicoverpa armigera* established from fenvalerate discriminating dose survivors from the Darling Downs area of southern Queensland in 1983/84. Colony 1 split at F1. Ordinate, log (LD50 × 100) (µg/30–40 mg larva) ± 95% confidence interval. Resistance factors above or below each confidence limit. Data from table 10.

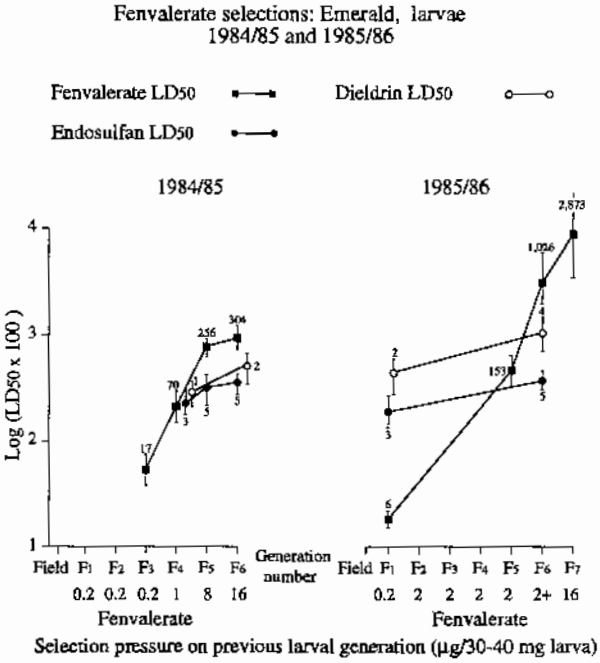


Fig. 23. Impact of fenvalerate selection on pyrethroid and cyclodiene resistance in larvae of two colonies of *Helicoverpa armigera* established from survivors from the Emerald fenvalerate screens, in 1984/85 and 1985/86. Ordinate, log (LD50 × 100) (µg/30–40 mg larva) ± 95% confidence interval. Resistance factors above or below each confidence limit. Data from table 11.

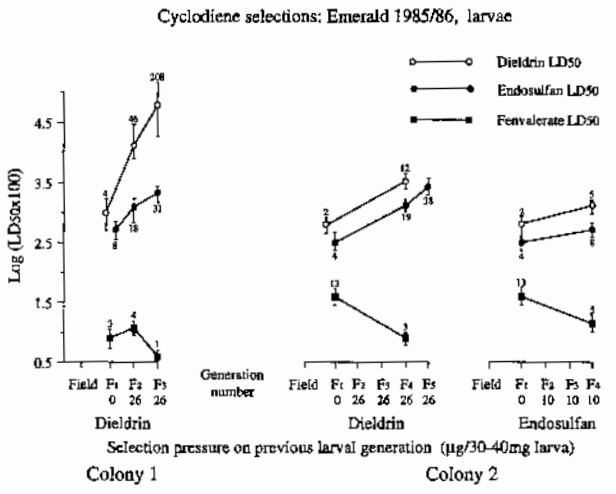


Fig. 24. Impact of larval cyclodiene selection on cyclodiene and pyrethroid resistance in larvae of two colonies of *Helicoverpa armigera* established from survivors from the 1985/86 Emerald fenvalerate screens. Colony 2 split at F1. Ordinate, log (LD50 × 100 (µg/30–40 mg larva) ± 95% confidence interval. Resistance factors above or below each confidence limit. Data from table 12.

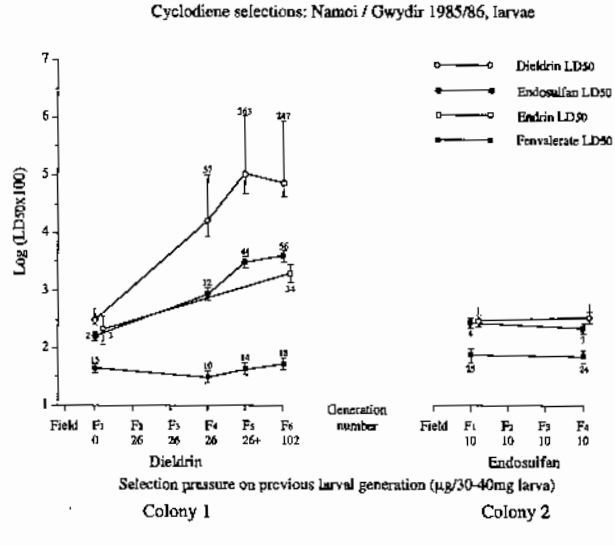


Fig. 25. Impact of larval cyclodiene selection on cyclodiene and pyrethroid resistance in larvae of two colonies of *Helicoverpa armigera* established from survivors from the 1985/86 Namoi/Gwydir fenvalerate screens. Ordinate, log (LD50 × 100 (µg/30–40 mg larva) ± 95% confidence interval. Resistance factors above or below each confidence limit. Data from table 13.

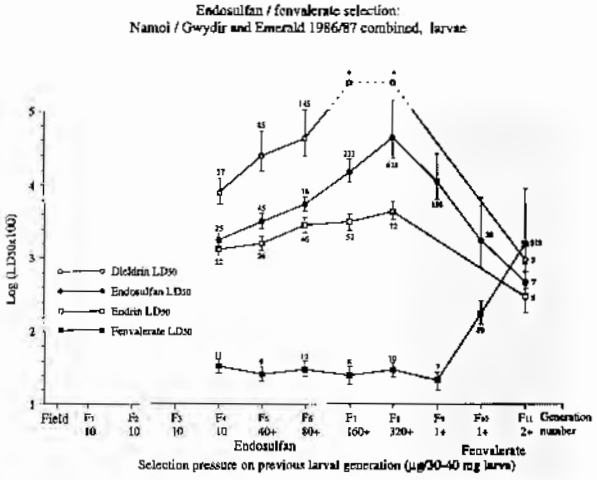


Fig. 26. Impact of larval endosulfan selection on cyclodiene and pyrethroid resistance in larvae of a colony of *Helicoverpa armigera* established from the pooled survivors from the 1986/87 Namoi/Gwydir and Emerald endosulfan screens. Selection switched to fenvalerate after the eighth generation. Ordinate, log ($LD_{50} \times 100$) (µg/30–40 mg larva) ± 95% confidence intervals. Resistance factors above or below each confidence limit. * = resistance factor not determinable. Data from table 14.

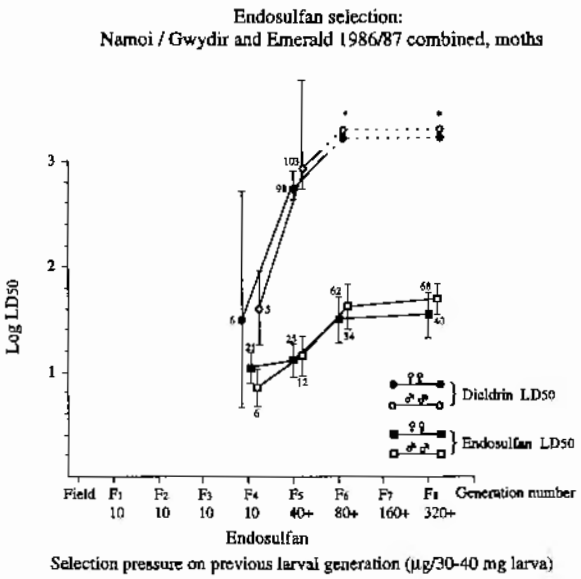


Fig. 27. Impact of larval endosulfan selection on cyclodiene resistance in adults of a colony of *Helicoverpa armigera* established from the pooled survivors from the 1986/87 Namoi/Gwydir and Emerald endosulfan screens. Ordinate, log LD_{50} (µg/200 mg standard 1 day old moth) ± 95% confidence intervals. Resistance factors above or below each confidence limit. * = resistance factor not determinable. Data from table 15.