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FINAL REPORT

"Organophosphate and carbamate resistance in *Helicoverpa armigera*"

DAN 104C

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Dr R.V. Gunning,
Principal Research Scientist,
NSW Agriculture
Tamworth Centre for Crop Improvement

NSW Agriculture

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Organophosphate and Carbamate resistance in *Helicoverpa armigera*

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CRDC Responsible Director (if known)
Dr Vic Edge

Organisation NSW Agriculture

Administrative contact Mr. G.D. Denny
Phone: (02) 6391 3219 E-mail: Fax: (02) 6391 3327
Postal Address 161 Kite Street
ORANGE
NSW, 2800

Project supervisor Mr. Dallas Gibb
Phone: (02) 6799 1500 E-mail: Fax: (02) 6793 1186

Principal Researcher Dr Robin Gunning
Phone: (02) 6763 1186 E-mail: rgunning@enternet.com.au Fax: (02) 6763 1222

ORGANOPHOSPHATE AND CARBAMATE RESISTANCE IN *HELICOVERPA ARMIGERA*

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SUMMARY

a) BACKGROUND TO THE PROJECT

Insecticide resistance in *Helicoverpa armigera* is a major threat to the economic production of cotton in Australia. So far, the effects of resistance in the cotton industry have been ameliorated by a resistance management strategy and the introduction of commercial BT transgenic cotton. However, the marginal performance in the field of transgenic cotton and large areas of non-transgenic cotton, has resulted in an increased reliance on conventional chemicals and ever increasing resistance and environmental problems associated with insecticide and conventional insecticides are likely to remain an important component of future *Helicoverpa* control strategies. Avoidance or minimisation of resistance can only be achieved by effective resistance monitoring and understanding underlying resistance mechanisms.

While new *Helicoverpa* spp. control chemicals continue to be slowly introduced into the resistance management strategy, the risk of the development of resistance to new insecticides, due to overuse, is extreme. It is necessary to keep the older insecticides, which are the mainstay for *Helicoverpa* control, working. Thus, it is essential that research to overcome resistance to older insecticides (pyrethroids, carbamates and organophosphates) is continued so that their efficacy is maintained or even improved against resistant *H. armigera*

b) PROJECT OBJECTIVES

- To monitor pyrethroid, endosulfan, organophosphate and carbamate resistance in *H. armigera* and *H. punctigera*. from all cotton areas and to refine resistance management strategies.

- Determine the genetic basis of carbamate in *H. armigera* and *H. punctigera* and organopbosphate resistance in *H. armigera*

- To develop and promote use of rapid biochemical techniques for the detection of organopbosphate and carbamate resistance in the field.

- To evaluate new insecticides for *Helicoverpa* control, establish baseline susceptibility data and investigate their inherent resistance potential.

All objectives of this project have been achieved.

c) RESULTS

(i) *Helicoverpa* resistance monitoring

Helicoverpa spp. eggs or larvae were collected during the cotton season, from each of the major cotton growing areas in NSW, Queensland and WA. *Helicoverpa armigera* were assayed for resistance to pyrethroids, endosulfan, carbamates (thiodicarb, methomyl), organophosphates (methyl parathion, profenofos and chlorpyrifos), amitraz, chlorfenapyr and spinosad. *Helicoverpa punctigera* were bioassayed with fenvalerate, endosulfan and methomyl. Results are disseminated to the cotton industry via the internet.

(ii) Selection for *H. armigera* carbamate resistance by ovicidal rates of methomyl

Ovicidal use of methomyl against *Helicoverpa* spp. The results clearly demonstrated that insecticide resistance in expressed in the black egg stage of *H. armigera*. Both larvicidal and ovicidal rates of methomyl selected for resistance in black eggs quite strongly. While it is not clear how laboratory data relates to the field, we must not assume that ovicidal use of methomyl has no selective effect on eggs.

(iii) Genetic basis of *H. armigera* carbamate resistance

Carbamate resistance in *H. armigera* , is, as a result of, an altered target site acetylcholinesterase, which is insensitive to inhibition by methomyl and thiodicarb. Genetic experiments, indicate that the resistance mechanism is caused by a single, incompletely dominant, autosomal gene . There are three genotypes homozygotes (RR) heterozygotes (RS), and susceptible (SS). Resistance is incompletely expressed in the heterozygotes giving rise to a lower resistance factor than homozygotes. The frequency of homozygotes (RR) in field populations are at a much lower frequency than predicted by population genetics . This indicate that there may be a lack of fitness of homozygotes in the field and could be very advantageous for resistance management.

(iv) Carbamate resistance in *H. punctigera*

H. punctigera that are carbamate resistant are widespread in the field. The resistance factor is approximately 12 fold. The resistance mechanism is an altered target site acetylcholinesterase esterase, insensitive to inhibition

by methomyl and thiodicarb. Genetic experiments indicated that resistance is a result of a single, incompletely dominant gene which is carried on the female sex determining chromosome.

(v) Organophosphate resistance in *H. armigera*
Selection of field *H. armigera* with profenofos achieved high levels of resistance to both profenofos (92 fold) and methyl parathion (52 fold) compared to the susceptible strain, however, strains were susceptible to chlorpyrifos. The resistance mechanism is an altered target site acetylcholinesterase esterase, insensitive to inhibition by the metabolites of methyl parathion and profenofos., but is still is susceptible to chlorpyrifos. Genetic experiments are incomplete, however, there are three genotypes homozygotes (RR) heterozygotes (RS), and susceptible (SS). Resistance is incompletely expressed in the heterozygotes giving rise to a lower resistance factor than homozygotes. Homozygous larvae have a severe fitness deficit.

(vi) Rapid Biochemical resistance detection
Studies have shown that pyrethroid, carbamate and organophosphate in Australian *H. armigera* resistance are as a result of biochemical resistance mechanisms. Pyrethroid resistance in Australian *H. armigera* is caused by a massive overproduction of esterase enzymes which detoxify pyrethroids by sequestration and hydrolysis. Esterase activity is correlated to resistance factor. Carbamate and organophosphate resistance in *H. armigera* are as a result of two different types of insensitive acetylcholine esterase (AChE). A result of resistance mechanism studies, has been the development of rapid biochemical methods for the detection of pyrethroid, carbamate resistance in Australian *H. armigera*. The methods are based on assays for resistance enzymes. Such methods can be utilised for laboratory based resistance detection, and field based, resistance detection kits have been produced and tested.

(vii) New control chemicals for *Helicoverpa*
Baseline data has been accumu;lated and pre-emptive resistance mechanism studies have been undertaken for new *H. armigera* control chemicals. *H. armigera* are showing some resistance to chlorfenapyr and a resistance mechanism has been identified.

(viii) Pyrethroid synergism - biochemical studies
The basis of pyrethroid synergism by piperonyl butoxide (PBO) and propargite (Comite®) is inhibition of the esterase enzymes which metabolise pyrethroids. Both synergists are only partial enzyme inhibitors so that use will select for populations which are more highly resistant to pyrethroids. Some organophosphates (such as ethion), on the other hand, are excellent esterase inhibitors and pyrethroid synergists.

(ix) Pyrethroid/esterase binding in *H. armigera*
Bioassay results show that all pyrethroids did not act identically on resistant *H. armigera* . Flucythrinate, fenvalerate and es-fenvalerate, were the least effective while permethrin, deltamethrin, bifenthrin, zeta-cypermethrin and alpha-cypermethrin were more toxic to resistant *H. armigera*,. Inhibition studies showed that this differences appears due to a differing ability of pyrethroids to bind to esterase enzymes. An examination of the structures of the pyrethroids, indicates marked differences in structure in those which interacted more readily with *H. armigera* esterases and those which bound less readily. The former group, are pyrethroids which contain a halogenated benzyl group. While in the latter group of pyrethroids (the benzyl ring was replaced by a dihalogenated aliphatic entity.

(x) Bifenthrin resistance in *H. armigera*
Until recently, pyrethroid resistant *H. armigera* were effectively susceptible to bifenthrin, but there been a considerable increase in the frequency of *H. armigera* larvae are resistant to bifenthrin over the last 3 years. Resistance is of a low order (~10 fold) and results from the evolution of additional esterase isoenzymes, which can bind to bifenthrin more efficiently. The resistance mechanism appears specific to bifenthrin.

(xi) Pyrethroid resistance in *H. punctigera*
Pyrethroid resistance can occur in *H. punctigera* and is a consequence of metabolism by esterase isoenzymes.

2. BACKGROUND TO THE PROJECT

Insecticide resistance in *Helicoverpa armigera* is a major threat to the economic production of cotton in Australia. So far, the effects of resistance in the cotton industry have been ameliorated by a resistance management strategy and the introduction of commercial BT transgenic cotton. However, the marginal performance in the field of transgenic cotton and large areas of non-transgenic cotton, has resulted in an increased reliance on conventional chemicals and ever increasing resistance and environmental problems associated with insecticide and conventional insecticides are likely to remain an important component of future *Helicoverpa* control strategies. Avoidance or minimisation of resistance can only be achieved by effective resistance monitoring and understanding underlying resistance mechanisms.

While new *Helicoverpa* spp. control chemicals continue to be slowly introduced into the resistance management strategy, the risk of the development of resistance to new insecticides, due to overuse, is extreme. It is necessary to keep the older insecticides, which are the mainstay for *Helicoverpa* control, working. Thus, it is essential that research to overcome resistance to older insecticides (pyrethroids, carbamates and organophosphates) is continued so that their efficacy is maintained or even improved against resistant *H. armigera*

3. PROJECT OBJECTIVES

- To monitor pyrethroid, endosulfan, organophosphate and carbamate resistance in *H. armigera* and *H. punctigera*. from all cotton areas and to refine resistance management strategies.
- Determine the genetic basis of carbamate in *H. armigera* and *H. punctigera* and organophosphate resistance in *H. armigera*
- To develop and promote use of rapid biochemical techniques for the detection of organophosphate and carbamate resistance in the field.
- To evaluate new insecticides for *Helicoverpa* control, establish baseline susceptibility data and investigate their inherent resistance potential.

All objectives of this project have been achieved.

4. METHODOLOGY

Methods used in this project this project have been a combination of bioassay, toxicological, biochemical and electrophysiological techniques. These techniques are the most appropriate methods to study insecticide resistance in *Helicoverpa* species.

5. RESULTS

a) Monitoring of resistance to conventional chemicals in *Helicoverpa* spp.

Helicoverpa spp. eggs or larvae were collected during the cotton season, from each of the major cotton growing areas in NSW, Queensland and WA. *Helicoverpa armigera* were assayed for resistance to pyrethroids, endosulfan, carbamates (thiodicarb, methomyl), organophosphates (methyl parathion, profenofos and chlorpyrifos), amitraz, chlorfenapyr and spinosad. *Helicoverpa punctigera* were bioassayed with fenvalerate, endosulfan and methomyl.

Results from each cotton growing district, are presented in the sections following.

(i) Macquarie Valley

H. armigera abundance data for the Macquarie Valley are presented in Fig. 1, the data clearly show that the Macquarie Valley does experience heavy *H. armigera* pressure.

Pyrethroids

Despite a high fenvalerate resistance frequency (~100%), over recent years, the resistance factor has been relatively low in the Macquarie Valley (Fig. 2). A low resistance factor means that, *H. armigera* are relatively susceptible to field rates of pyrethroids and this has been reflected in good field control of *H. armigera* with pyrethroids. A low resistance factor is as a consequence of adherence to a short (stage 2), pyrethroid spray window in the Macquarie Valley. A reduction in selection pressure causes a decline in pyrethroid resistance and *H. armigera* are usually susceptible to field rates of pyrethroids for the commencement of each season. Restriction of pyrethroid use in the Macquarie Valley has greatly reduced selection pressure on *H. armigera*., thus preventing build-up of highly resistant populations and the control problems experienced in other cotton growing areas. Nevertheless, recent spring use of pyrethroids against cutworms and armyworm in cereal and grain legumes in 1997/98. has increased the selection pressure and early season resistance factor. The frequency of *H. armigera* , resistant to bifenthrin increased in the Macquarie Valley 1996 - 99 (Fig. 3) , but the resistance factor remains low, (~ 5 fold).

Carbamate resistance

Carbamate resistance in the Macquarie Valley appears to have stabilised in recent years, although the frequency of *H. armigera* resistant to methomyl and thiodicarb is high (~ 80 %) (Fig. 4). The proportion of individuals which are homozygous for resistance has remained low (~10%) and it would appear that the embargo on using carbamates until stage 3 in the Macquarie Valley is effective.

Organophosphate resistance

Frequency of *H. armigera* resistant to profenofos and methyl parathion has increased (up to 30%), in the Macquarie Valley in 1998/99, (Fig. 5) and poor control was reported on some farms. It would appear that the practice of mixing profenofos and methyl parathion is accelerating selection for resistance. *H. armigera* in the Macquarie Valley, remain susceptible to chlorpyrifos.

Endosulfan resistance

In common with most *H. armigera* populations from cotton, the endosulfan resistance frequency has declined in recent years (Fig. 6). The endosulfan resistance factor also is very low (< 5 fold).

Other compounds

No significant resistance to amitraz, chlorfenapyr or spinosad were detected in the Macquarie Valley.

Resistance in <u>Helicoverpa punctigera</u>

H. punctigera resistant to fenvalerate, endosulfan and methomyl were detected in the Macquarie Valley. While resistance to fenvalerate and endosulfan is rare, methomyl resistance is quite common.

(ii) Upper Namoi

Pyrethroid Resistance

Fenvalerate resistance frequency in the Upper Namoi *H. armigera* populations has been ~100%. for a number of years and the resistance factor has increased approximately 5 fold, (Fig. 7). Fenvalerate is a highly resisted pyrethroid compared to other pyrethroids, the resistance factors for commercially available pyrethroids deltamethrin, a-cypermethrin, bifenthrin etc. are lower, but increasing.

While lack of egg numbers did not permit extensive monitoring of bifenthrin resistance in the Upper Namoi., as in other areas, the resistance frequency is increasing over time but the resistance factor is remains low ~ 5 fold.

Carbamate resistance

Since 1996, carbamate resistance appears to have become fixed in Upper Namoi *H. armigera* populations (Fig. 8) and by 1998/99, resistant homozygotes comprised about 20 % of the population.

Organophosphate resistance

The frequency of *H. armigera* resistant to profenofos and methyl parathion is low in the Upper Namoi. and there is here is no resistance to chlorpyrifos (Fig. 9).

Endosulfan resistance

In the Upper Namoi, endosulfan resistance frequency has declined overall and the resistance factor is very low (< 5 fold).

Resistance to other compounds

H. armigera showed no resistance to amitraz and spinosad, but some survival of chlorfenapyr (5%), in one sample during 1998/99.

Resistance in Helicoverpa punctigera
In the Upper Namoi, some *H. punctigera* were resistant to fenvalerate, endosulfan and methomyl. Resistance to fenvalerate and endosulfan was rare. Methomyl resistance is quite common.

(iii) Lower Namoi
Data for 1996/97 - 1997/98 is in shown in Figs. 10 - 13. *Helicoverpa* egg collection problems prevented accumulation of much data in 1998/99.

Pyrethroid resistance
Approximately 100% of *H. armigera* were resistant to fenvalerate, however resistance factor varied seasonally according to pyrethroid use. In 1996/97, the resistance factor rose to 40 fold, which is consistent with season wide pyrethroid usage. When pyrethroids were removed from stage 1 in 1997/98,, resistance factor to dropped to 20 fold but increased with pyrethroid use during Stages 2 and 3. Resistance to a fenvalerate/piperonyl butoxide mix increased, with fenvalerate resistance factor. Bifenthrin resistance frequency increased to approximately 60% during 1997 - 1998. (Fig 10)

Carbamate resistance
Carbamate (methomyl and thiodicarb) resistance frequency and proportion of resistant homozygotes, increased during 1996 - 1998 , (Fig 11), although there was a district increase in susceptibility in response to a stage 1 embargo on the use of all carbamates in 1996/97.

Organophosphate resistance
The frequency of *H. armigera* resistant to profenofos and methyl parathion was low overall and even declined during 1997/98 and there was resistance to chlorpyrifos (Fig 12).

Endosulfan resistance
During 1996 - 1998, the endosulfan resistance frequency in the Namoi Valley was reasonably stable at ~ 50%, Fig 13). and the resistance factor was very low (< 5 fold).

(iv) Insecticide Resistance in the Gwydir Valley
Pyrethroid Resistance
Fenvalerate resistance frequency was ~ 100% in the Gwydir Valley, during 1996 - 1999, (Fig. 14). Fenvalerate resistance factor, showed seasonal fluctuations, from a baseline of 25 - 30 fold, rising to peaks of 30 - 40 fold during periods of intensive pyrethroids use. The resistance factor reached approximately 60 fold in March 1999, in response to extreme selection pressure. An increasing resistance factor was accompanied by higher resistance to fenvalerate piperonyl butoxide mixes. Fenvalerate is a very highly resisted pyrethroid compared to other pyrethroids Resistance factors for other commercially available pyrethroids deltamethrin, a-cypermethrin, bifenthrin etc. were lower, but are increasing Frequency of *H. armigera* resistant to bifenthrin has increased from 26 - ~ 50 % over 3 years, in the Gwydir Valley (Fig. 15), but the resistance factor remains low (~ 5 fold).

Carbamate resistance
Frequency of *H. armigera* resistant to carbamates has increased in the Gwydir Valley and is currently, 80 - 90 %. They are largely heterozygous for resistance (Fig 16).

Organophosphate resistance
During 1996 - 1999, the frequency of *H. armigera* resistant to profenofos and methyl parathion was almost undetectable in the Gwydir Valley and there was no resistance to chlorpyrifos (Fig 17).

Endosulfan resistance
Endosulfan resistance frequency has declined in the Gwydir Valley (Fig 18) . The endosulfan resistance factor is very low (< 5 fold).

Resistance in Helicoverpa punctigera
H. punctigera resistant to fenvalerate, endosulfan and methomyl were detected in the Gwydir Valley. Resistance to fenvalerate and endosulfan is rare, but methomyl resistance is quite widespread.

(v) Insecticide resistance in south-east Queensland (Darling Downs and Burnett areas)
Pyrethroid Resistance
Fenvalerate resistance frequency was ~ 100% in SE Queensland during 1996 - 1999, the resistance factor averaged ~50 fold in the Darling Downs (Fig. 19), while in the Burnett , the resistance factor was a little lower

(~30 fold), (Fig 20). Fenvalerate was a very highly resisted pyrethroid compared to other pyrethroids Resistance factors for commercially available pyrethroids deltamethrin, a-cypermethrin, bifenthrin etc. ,were lower, but are increasing. Bifenthrin resistance frequency increased from ~ 10 to 50 % during 1996 - 1999 in the Darling Downs (Fig 21), but the resistance factor was low ~ 5 fold.

Carbamate resistance

The carbamate (methomyl and thiodicarb) resistance frequency increased to virtually 100% in the Darling Downs area (Fig 22), with frequency in the Burnett only slightly lower (Fig 23). A high frequency of homozygotes in the Darling Downs (40 %) at the end of the 1998/99 season, was probably caused by undisciplined use of carbamates . Homozygote levels in the Burnett were somewhat lower (`~20 %).

Organophosphate resistance

In spite of extensive use of profenofos and methyl parathion in the Darling Downs resistance frequency has not greatly altered (~20%) and there is no resistance to chlorpyrifos (Fig 24).

Endosulfan resistance

Endosulfan resistance frequency has lesseoed in the Darling Downs, since 1996. (Fig. 25).

Resistance in Helicoverpa punctigera

We detected *H. punctigera* resistant to fenvalerate, endosulfan and methomyl, while fenvalerate and endosulfan resistance was rare, methomyl resistance is quite common.

(vi) Insecticide resistance in Emerald

Pyrethroid Resistance

Fenvalerate resistance is appears fixed in Emerald, *H. armigera* populations (Fig 26). During 1996/97, the fenvalerate resistance factor rose to approximately 60 fold, presumably in response to extreme selection pressure. While levels in the following two seasons were lower, a resistance factor of 20 - 30 fold is still of concern for resistance management. Fenvalerate is a very highly resisted pyrethroid compared to other pyrethroids Resistance factors for commercially available pyrethroids deltamethrin, a-cypermethrin, bifenthrin etc. were lower, hut are increasing.

Bifenthrin resistance has steadily increased in Emerald since 1996. (Fig 27). By early 1999, approximately 70% of the population were resistant, however, the resistance factor is low ~ 5 fold.

Carbamate resistance

Since 1996, the frequency of methomyl and thiodicarb resistance in *H. armigera* populations has averaged ~ 80% (Fig. 28). The frequency of *H. armigera*, homozygous for resistance, was ~ 20%. Homozygotes have shown a marked tendency to increase rapidly during periods of carbamate use and the augmentation in early 1999, is of some concern.

Organophosphate resistance

Throughout, 1996 - 1999, profenofos and methyl parathion resistance frequency in Emerald populations of *H. armigera* (Fig 29),, has been higher than populations from other areas These levels, are presumably a response to extreme selection pressure and are of great concern. As yet, there is no resistance to chlorpyrifos.

Endosulfan resistance

In Emerald, endosulfan resistance frequency in Emerald populations has declined overall from 70 -to 30 %) since 1996, (Fig 30).The endosulfan resistance factor is also very low (< 5 fold).

Resistance to other compounds in H. armigera

Bioassays were conducted using amitraz, spinosad and chlorfenapyr on Emerald *H. armigera* (Fig 31). There were no signs of resistance development to amitraz and spinosad. There was, however, some survival (up to 5%), with chlorfenapyr.

Resistance in Helicoverpa punctigera

H. punctigera which were resistant to fenvalerate, endosulfan and methomyl in Emerald . Resistance to fenvalerate and endosulfan was rare. Methomyl resistance, however, is quite prevalent.

(vii) Insecticide resistance in St George

Pyrethroid Resistance

Fenvalerate resistance frequency, in St George populations of *H. armigera*, has been fixed at ~100% since 1996 (Fig. 32). The resistance factor averaged ~ 30 fold during the period, although in 1996, it approached 60 fold. More recently, there is evidence that selection pressure from excessive pyrethroid use has increased the

resistance factor. Fenvalerate is a very highly resisted pyrethroid compared to other pyrethroids. Resistance factors for commercially available pyrethroids deltamethrin, a-cypermethrin, bifenthrin etc. are lower, but are increasing. Over the last 3 years, the frequency of *H. armigera* resistant to bifenthrin has increased to ~ 70 % (Fig 33), however the resistance factor is very low (`5 fold).

Carbamate resistance
Since 1996, carbamate (methomyl and thiodicarb), resistance frequency in St George *H. armigera* populations has increased from 60 to almost 100% (Fig. 34). The proportion of resistant homozygotes has also increased to ~ 30%. Restrictions on early season carbamate use in 1996/97 were effective in containing homozygote frequency, however, relaxation of limitations has led to an increase.

Organophosphate resistance
H. armigera populations in St George showed a higher frequency of *H. armigera* resistant to profenofos and methomyl parathion than most other areas, (Fig 35). Extreme selection pressure from the use of pyrethroid / organophosphate mixes is of concern There is no resistance to chlorpyrifos (Fig 35).

Endosulfan resistance
Since late 1996, the proportion of St George *H. armigera* resistant to endosulfan has overall, declined, although the endosulfan rose again, sharply, in early 1999 (Fig 36). The endosulfan resistance factor was very low (< 5 fold).

Resistance to other compounds in H. armigera
In 1998/99, there was no resistance to either amitraz or spinosad, however some resistance to chlorfenapyr (~ 10%) was detected, (Fig 37).

Resistance in Helicoverpa punctigera
In common with other cotton growing areas, St Goerge*H. punctigera* were resistant to fenvalerate, endosulfan and methomyl. Resistance to fenvalerate and endosulfan was rare but methomyl resistance was quite common.

(viii) Insecticide resistance in Theodore/Biloela
Pyrethroid Resistance
Fenvalerate resistance frequency in *H. armigera* populations from Theodore and Biloela, has been approximately 100% since 1996, (Fig 38). The resistance factor currently averages ~ 30 fold. the frequency of *H. armigera* resistant to bifenthrin has increased from ~ 20 to 60 %), (Fig 39) but the resistance factor is low ~ 5 fold.

Carbamate resistance
The frequency of *H. armigera* resistant to methomyl and thiodicarb has increased to almost saturation since 1995/96. The frequency of resistant homozygotes, has remained quite low, averaging ~ 20% (Fig. 40).

Organophosphate resistance
The frequency of *H. armigera* resistant to profenofos/methyl parathion is low (~10%) and, there is no resistance to chlorpyrifos (Fig 41).

Resistance in Helicoverpa punctigera
In common with other cotton growing areas, we detected *H. punctigera* which were resistant to fenvalerate, endosulfan and methomyl. Resistance to fenvalerate and endosulfan is rare. Methomyl resistance, however, is quite common.

(ix) Insecticide resistance in the Macintyre Valley
Pyrethroid resistance
During 1996 - 1999 , fenvalerate resistance frequency in Macintyre Valley populations of *H. armigera* was virtually 100% and the resistance factor averaged ~ 30 fold, (Fig 42). The frequency of *H. armigera* resistant to a fenvalerate/piperonyl butoxide mix averaged 50%.
The bifenthrin resistance frequency in *H. armigera* rose from ~20% to 60% from 1996 - 1999., however, the resistance factor did not exceed 5 fold, (Fig 43).

Carbamate resistance
During 1996 - 1999, carbamate (methomyl and thiodicarb) resistance frequency in *H. armigera* has increased from ~ 50 % to almost 90%, (Fig 44). The proportion of resistant homozygotes has averaged ~ 15 %.

Organophosphate resistance
Profenofos and methyl parathion resistance in Macintyre *H. armigera* populations has remained relatively low (~10%) during 1996 - 1999, (Fig. 45). There was no resistance detected to chlorpyrifos.

Endosulfan resistance
Between 1996 and 1999, endosulfan resistance frequency has declined in Macintyre Valley *H. armigera* populations (Fig. 46).

Other Compounds
No resistance to amitraz or spinosad was detected in Macintyre Valley *H. armigera* populations, however in Feb. 1999, ~ 15% of *H. armigera* were resistant to chlorfenapyr, (Fig. 47).

(j) Insecticide resistance at Bourke

Insecticide resistance in *H. armigera* and *H. punctigera* populations from Bourke were monitored for first time in 1998/99. Results indicated that resistance levels to most insecticides are very similar to other populations in NSW and Qld. (Figs. 48,49).

(x) Discussion of *Helicoverpa* resistance monitoring data

Pyrethroids

Pyrethroid resistance situation has deteriorated in most cotton areas. Resistance levels have generally increased since 1996 . Areas of particular concern are the darling Downs, Macintyre Valley, Gwydir Valley and Bourke areas. Reasons for loss of efficacy are primarily, overuse of pyrethroids. However, inadvertent selection of the first generation of *H. armigera* hy use of pyrethroids on winter cereals and spring grain legumes to control cutworm and armyworms is becoming increasingly important. as a source of selection pressure. Recommendations to increase pyrethroid efficacy are as follows.

- A pyrethroid use window (similar to that in the Macquarie Valley) be introduced in all cotton areas. The window should be no longer than abnut 5 weeks (Stage 2 and early Stage 3). Maximum of 3 sprays in total (including hifenthrin and pyrethroid/piperonyl hutoxide mixes) No pyrethroids should he used be used in stage 1 and late stage 3.

- While currently used pyrethroid/organophosphate mixes are ineffective for pyrethroid synergism, there are much better organophosphate synergists which might he used (see following sections) Registrations of such products need to be encouraged.

- Other OP's Stages 2 and 3, no more than 2 sprays of each. Not to be mixed with pyrethroids.

Carbamates
The carbamate resistance situation has reacbed a difficult position, prohahly caused the use of ovicidal rates of methomyl, a lack of disipline in carhamate use.
Recomendations:

- Carbamates be used, at larvicidal rates, in Stage 3 only.

- Negotiation with growers of other summer crops to ensure consistency of carhamate use with all insecticide users.

Endosulfan

Endosulfan resistance appears to be declining and the resistance factor seldon exceeded 5 fold. I do not consider that endosulfan use patterns should be maintained.

Amitraz

There is little indication that any significant resistance is developing to amitraz and I do not think any changes in use are required here. However the manufactuers need to he discouraged from promoting their product as a synergist for everything!

Organophosphates

- OP sprays need to have a strict number limit.

- Mixtures of OP's have to be banned.

- An OP usc window.

New Products
There need to be limits on the number of sprays.

Helicoverpa punctigera
There has been an explosion in the field, of *H.punctigera* resistant to carbamates (methomyl and thiodicarb). The resistance is caused by an insensitive form of acetlycholinesterase. It is probable that use of methomyl (at ovicidal rates) in Stage 1 is the cause.

- Carbamates should be kept out of Stage 1 and left to Stage 3.

b) Carbamate resistance in *H. armigera*

(i) Selection for resistance by ovicidal rates of methomyl

Introduction
Carbamate (methomyl and thiodicarb), resistance is expressed in both larval and adult stages of *H. armigera*. Resistance, due to an insensitive target site in the nervous system, is not shown in the egg stage until the embryonic nervous system begins to function. Methomyl is an important ovicide on cotton and it was important to determine at which stage of egg development resistance is expressed.

Insects
Susceptible and selected resistant *H. armigera* strains were used in these experiments. were a susceptible strain The selected strain was approximately 50% heterozygous and 50% homozygous for resistance.

Methods
30 white, brown or black eggs were placed on cotton leaves and sprayed via the potter tower with average ovicidal and larvicidal rates of methomyl (0.75 and 2.1 L/ha). The leaves were allowed to dry and placed in petri dishes. (The petiole was allowed access to water using "florist foam"). The dishes were held in natural light at 25°C. Controls sprayed were carried. Mortality was assessed 48 h after controls had hatched. Numbers of unhatched eggs, dead larvae and live larvae were recorded and corrected for any control mortality. Experiments were replicated 3 times. Survivors were placed on fresh food until they were 3-4 mg and were then assayed for carbamate resistance using biochemical methods to detect an insensitive acetylcholinestase. Proportions of the populations, susceptible, heterozygous or homozygous for resistance were recorded.

Table 1
Selection for carbamate resistance in *H. armigera* eggs with ovicidal and larvicidal rates of methomyl

Strain	egg colour	unhatched eggs %	dead larvae %	live larvae %	resistance status survivors
Ovicidal rate					
sus.	white	100	0	0	-
	brown	100	0	0	-
	black	92	4	4	SS
resist.	white	100	0	0	-
	brown	100	0	0	-
	black	13	4	83	RS (55%) RR (45%)
Larvicidal rate					
sus.	white	100	0	0	-
	brown	100	0	0	-
	black	100	0	0	-
resist.	white	100	0	0	-
	brown	91	0	9	RR (100%)
	black	2	0	98	RR (100%)

Results and Discussion

The results (Table 1) clearly demonstrated that insecticide resistance in expressed in the black egg stage of *H. armigera*. Both larvicidal and ovicidal rates of methomyl selected for resistance in black eggs quite strongly but selection pressure was greater using larvicidal rates. While it is not clear how laboratory data relates to the field, we must not assume that ovicidal use of methomyl has no selective effect on eggs.

(ii) The genetic basis of carbamate resistance in *H. armigera*

Carbamate resistance in *H. armigera* , is, as a result of, an altered target site acetylcholinesterase, which is insensitive to inhibition by methomyl and thiodicarb (Fig 50 , 51). The altered enzyme is > 500 times less sensitive to methomyl and thiodicarb than the susceptible enzyme has differing kinetic properties to the susceptible form (Table 2). Backcrossing experiments, bioassay and biochemical assays indicate that the resistance mechanism is caused by a single, incompletely dominant, autosomal gene (Table 3). There are three genotypes RR, RS, and SS (Fig. 52). Resistance is incompletely expressed in the heterozygotes giving rise to a lower resistance factor than homozygotes. Carbamate resistance frequency in *H. armigera* cotton populations is high and gives little indication of a fitness deficit, however, the frequency of homozygotes in field populations are at a much lower frequency than predicted by Hardy-Weinberg equation for population genetics (Fig. 53). These data indicate that there may be a lack of fitness of homozygotes in the field and could be very important for resistance management.

A resistance mechanism, detectable by colorimetic analysis has given the opportunity for rapid, field-based, biochemical resistance detection.

Table 2

Kinetic parameters of AChE from methomyl-susceptible and resistant *H. armigera* larvae.

	K_m[b] (μM)	V_{max}[b] ($mOD^{-1} min^{-1} mg^{-1}$)	k_i[c] ($M^{-1} min^{-1}$)
Susceptible	1.8(0.1)	6..8 (0.1)	5.3 (0.2) x 10^4
Resistant	0.3 (0.01)	14.5 (0.2)	1.3 (0.1) x 10^3

[a]Values are the result of three independent determinations (± SEM)
[b] Substrate, acetylthiocholine iodide.
[c] methomyl

Table 3

Genetics of insensitive AChE methomyl resistance in *H. armigera*

Cross	Ratio SS : RS : RR	Predicted (autosomal)
RS x RS	0.95 : 2..1 : 0.95	1 : 2 : 1
RR (f) xSS (m)	0 : 1 : 0	0 : 1 : 0
RR (m) x SS (f)	0 : 1 : 0	0 : 1 : 0

c) Mechanism and genetic basis of carbamate resistance in *Helicoverpa punctigera.*

Introduction

Resistance monitoring bas shown that methomyl resistance is relatively common in field populations of *H. punctigera* in NSW and Queensland. Ther have been recent,(Dec. 1999), eports of carbamate failures against *H. punctigera.*

Methods

Tbe colony of methomyl susceptible *H. punctigera* was derived from moths collected via a light trap at Tamwortb, NSW. Populations of metbomyl resistant *H, punctigera* were obtained from egg collections made on carbamate treated cotton from tbe Macquarie Valley. methomyl susceptibility was monitored using a rapid discriminating dose technique (1.0 µg/larva). Survivors were reared and two subsequent generations subjected to further selection with doses that killed approximately 70 % of the population, (12.5 µg/larva , 10 x the discriminating dose). Insecticide used was technical grade methomyl, it was bioassayed by topical application on 3rd instar larvae.

Activity of acetylcholinestase (AChE) in *H. punctigera* was measured by the method of Ellman *et. al* , in which the hydrolysis of the substrate analogue acetylthiocholine iodide (ATChI)is measured colorimetically by the absorbance of 2-nitro - 5- thiobenzoate at 405 nm, after the reaction of 5,5'- dithiobis (2-nitrobenzoate) (DTNB) with the liberated thiocholine. Assays were done in a microplate. Mass homogenates of 10, *H. punctigera* larvae (each 3-4 mg) were made in 200 µl 0.01 M phosphate buffer, pH 7.5, containing 0.05% Triton X-100 and 10 µl aliquots placed in 96 well microplates. ATChI and DTNB solutions (in buffer) were added (100 µl each), to give final concentrations of 0.5 mM and 0.05 mM respectively. Activity was measured continuously in a Bio-Rad microplate reader for 20 mins, utilising kinetic collector software to fit linear regressions to the kinetic plots. Activity was usually linear up to an absorbance limit of 50 m OD.

For inhibition studies, varying concentrations of methomyl stock solutions prepared in acetone were evaporated and the insecticide re-dissolved in the ATCh I /buffer solution. 100µl was then added to the wells containing homogenate and DTNB with an eight-channel multipipette. Wells containing 100µl of ATChI in buffer served as uninhibited controls. Enzyme activity in the presence of insecticides was calculated as a percentage of the corresponding uninhibited rate. Curves of enzyme activity versus final insecticide concentratioo in the wells were plotted.

Values of K_m and V_{max} were determined at 25°C from uninhibited AChE activities, measured over 10 mins for 10 ATChI concentrations ranging from 8×10^{-5} M to 3×10^{-2} M. K_m and V_{max} values were calculated from the Michaelis-Menten equation using EnzFitter software. Bimolecular rate constants (K_i) for hydrolysis of ATChI inhibited by methomyl were calculated using a similar approach, by non-linear regression using EnzFitter software.

Results

Selection with methomyl produced a strain *H. punctigera* that was 12.3 fold resistant to methomyl (Table 4). Research shows that, *H. punctigera* had developed an altered target site acetylcholinesterase esterase, which was insensitive to inhibition hy methomyl. (Fig. 54). Backcrossing experiments indicated that resistance is a result of a single, incompletely dominant gene which is carried on the female sex determining chromosome (Table 5). Altered acetylcholinesterase in *H. punctigera* differs in affinity to methomyl from AChE in carbamate resistant *H. armigera* and resistance in hoth species is not caused by the same altered form of acetylcholinesterase or mediated by the same gene.(Tahles 3, 6)

Table 4

Toxicity of methomyl to susceptible and resistant strains of 3rd instar, *Helicoverpa punctigera*

Strain	Slope	LD_{50} µg/larva	Fiducial limits	χ^2	RF
susceptible	3.4	0.048	0.032 - 0.069	0.34	-
resistant (sel)	2.8	0.59	0.46 - 0.77	0.46	12.. 3

Table 5

Genetics of insensitive AChE methomyl resistance in *H. punctigera*

Generation	Ratio SS : RS : RR	Predicted (autosomal)	Predicted (sex linked) X chromosome
RR (f) xSS (m)	0 : 1.1 : 0.9	0 : 1 : 0	0 : 1 : 1
RR (m) x SS (f)	0.98: 1.02 : 0	0 : 1 : 0	1 : 1 : 0

Table 6

Kinetic parameters[a] of AChE from methomyl-susceptible and resistant *H. punctigera* larvae.

	K_m[b] (µM)	V_{max}[b] ($mOD^{-1} min^{-1} mg^{-1}$)	k_i[c] ($M^{-1} min^{-1}$)
Susceptible	0.1(0.02)	3.6 (0.1)	1.4 (0.2) x 10^4
Resistant	0..04	7.8 (0.2)	4.5 (0.2) x 10^2

[a]Values are the result of three independent determinations (± SEM)
[b] Substrate, acetylthiocholine iodide.
[c] methomyl

d). Organophosphate resistance in *H. armigera*

Introduction

Organophosphates have long been used for *H. armigera* control and are effective larvicides. However, as resistance to pyrethroids, endosulfan and carbamates has increased, so has the use of chemicals such as the organophosphates on cotton, particularly late in the cotton growing season. Organophosphates currently used against *H. armigera* on cotton in Australia are profenofos, methyl parathion and chlorpyrifos. Low frequencies of *H. armigera* resistant to profenofos have been recorded since 1985 but this resistance was rarely expressed as field control problems. However, increased organophosphate use in recent years has resulted in some reports of poor field control.

Methods

The colony of organophosphate susceptible *H. armigera* was derived from moths collected via a light trap at Tamworth, NSW (Feb. 1997). Populations of organophosphate resistant *H, armigera* were obtained from egg collections made on profenofos treated cotton from South-east Queensland (Feb. 1997). Profenofos susceptibility was monitored using a rapid discriminating dose technique(1.25 mg/larva). Survivors were reared and two subsequent generations subjected to further profenofos selection with doses that killed approximately 70 % of the population, (12.5 mg/larva , 10 x the discriminating dose), and then 62.. 5 mg/larva (50 x the discriminating dose). The progeny of selection were then used in the following experiments

Insecticides used were technical grade methyl parathion (99%, Bayer Australia), methyl paraoxon (99.9%, Bayer, Australia),profenofos (89.3% Ciba Geigy), chlorpyrifos (99%, Dow Elanco, Australia) and

chlorpyrifos-oxon (98.5%, Dow Elanco, Australia). Methyl parathion, profenofos and chlorpyrifos were bioassayed by topical application.

Activity of acetylcholinestase (AChE) in *H. armigera* was measured by the method of Ellman *et. al* , in which the hydrolysis of the substrate analogue acetylthiocholine iodide (ATChI)is measured colorimetically by the absorbance of 2-nitro - 5- thiobenzoate at 405 nm, after the reaction of 5,5'- dithiobis (2-nitrobenzoate) (DTNB) with the liberated thiocholine. Assays were done in a microplate. Mass homogenates of 10, *H. armigera* larvae (each 3-4 mg) were made in 200 µl 0.01 M phosphate buffer, pH 7.5, containing 0.05% Triton X-100 and 10 µl aliquots placed in 96 well microplates. ATChI and DTNB solutions (in buffer) were added (100 µl each), to give final concentrations of 0.5 mM and 0.05 mM respectively. Activity was measured continuously in a Bio-Rad microplate reader for 20 mins, utilising kinetic collector software to fit linear regressions to the kinetic plots. Activity was usually linear up to an absorbance limit of 50 m OD.

For inhibition studies, varying concentrations of methyl paraoxon, profenofos and chlorpyrifos-oxon stock solutions prepared in acetone were evaporated and the insecticide re-dissolved in the ATCh I /buffer solution. 100µl was then added to the wells containing homogenate and DTNB with an eight-channel multipipette. Wells containing 100µl of ATChI in buffer served as uninhibited controls. Enzyme activity in the presence of insecticides was calculated as a percentage of the corresponding uninhibited rate. Curves of enzyme activity versus final insecticide concentration in the wells were plotted.

Values of K_m and V_{max} were determined at 25°C from uninhibited AChE activities, measured over 10 mins for 10 ATChI concentrations ranging from 8×10^{-5} M to 3×10^{-2} M. K_m and V_{max} values were calculated from the Michaelis-Menten equation using EnzFitter software. Bimolecular rate constants (K_i) for hydrolysis of ATChI inhibited by methyl para-oxon were calculated using a similar approach, by non-linear regression using EnzFitter software.

Results

Results of bioassays are shown in Table 7 Selection of field *H. armigera* with profenofos achieved high levels of resistance to both profenofos (92 fold) and methyl parathion (52 fold) compared to the susceptible strain. Both strains were susceptible to chlorpyrifos.

The inhibitory effects of methyl paraoxon on AChE activity from mass homogenates of resistant and susceptible *H. armigera* are shown in Fig. 55 . AChE from the resistant strain was clearly less sensitive to inhibition by methyl paraoxon, compared to the susceptible strain. Based on the concentration of methyl paraoxon required to inhibit all enzyme activity in the susceptible strain (IC_{100} = 33µM), the resistant AChE was approximately 8 times less sensitive to inhibition. We were unable to determine whether resistant AChE was less sensitive to inhibition by profenofos because of the limited solubility of profenofos. Profenofos is also known to be a poor AChE inhibitor, unless activated to its S-oxide form· Chlorpyrifos-oxon was a potent AChE inhibitor in both resistant and susceptible strains (Fig 56).

Kinetic parameters of the resistant and susceptible AChE's are shown in Table 8 Differences in K_m and V_{max} between the resistant and susceptible strains suggest that there are different forms of AChE. In the resistant population, the K_m was almost two times greater than the susceptible strain, possibly reflecting a decrease in the affinity of the insensitive enzyme. Bimolecular rate constants (k_i) which provide a reliable measure of AChE insensitivity to inhibition by methyl para-oxon were approximately 100 times higher in the susceptible strain than the resistant strain, showing considerable insensitivity to this inhibitor. Uninhibited AChE activity was somewhat reduced in the resistant strain compared to the susceptible strain and this is consistent with other work which indicates that target site resistance is associated with a change in the kinetic properties of the enzyme.

Table 7
The response of third instar *Helicoverpa armigera* larvae to methyl parathion, profenofos and chlorpyrifos.

Strain	LD_{50} mg/larva	Fiducial limits	Slope	X^2	RF
profenofos					
susceptible	0.14	(0.11 - 0.16)	3.6	2.2	-
resistant	13.0	(11.9 - 15.2)	3.7	2.1	92
methyl parathion					
susceptible	0.39	(0.30 - 0.48)	4.3	2.5	-
resistant	20.3	(18.5 - 23.1)	4.5	1.9	52
chlorpyrifos					
susceptible	3.7	(3.5 - 3.9)	4.5	1.7	-
resistant	3.8	(3.6 - 4.0)	4.4	1.8	-

Table 8

Kinetic parameters[a] of AChE from organophosphate-susceptible and resistant *H. armigera* larvae.

	K_m[b] (μM)	V_{max}[b] ($mOD^{-1}min^{-1}mg^{-1}$)	k_i[c] (M^{-1} min^{-1})
Susceptible	15 (2.8)	14.2 (0.3)	3.8 (0.3) x 10^5
Resistant	45 (2.7)	8.2 (0.2)	3.6 (0.2) x 10^3

[a]Values are the result of three independent determinations (± SEM)
[b] Substrate, acetylthiocholine iodide.
[c]methyl para-oxon

Discussion

Organophosphate and carbamate insecticides share a common target site, organophosphate insensitive, however, There is no association between different forms of insensitive AChE's causing organophosphate and carbamate resistance and no cross resistance.

Insecticide use against Australian *H. armigera* is subject to a resistance management strategy. While organophosphate resistance has been known for some time in *H. armigera*, there has recently been an expanded use of profenofos, methyl parathion and chlorpyrifos on cotton, due to poor control with other insecticides and the variable performance of transgenic cotton. Profenofos resistance *H. armigera* has increased , *H. armigera* remain susceptible to chlorpyrifos. Management strategies have been proposed, by limiting the use of organophosphates. Our studies indicate that this resistance is associated with a real fitness deficit, resulting in slower larval growth, which combined with the judicious use of chlorpyrifos as an alternative insecticide, may further retard the development of further field resistance problems.

A resistance mechanism, detectable by colorimetic analysis gives the opportunity for rapid field-based biochemical resistance detection. Such methods are already in use for the rapid diagnosis of pyrethroid and carbamate resistance in *H. armigera*. The detection of this resistance mechanism may lead to a rapid field based detection of OP resistance in *H. armigera*.

e) Genetic basis of organophosphate resistance in *H armigera*

In *H. armigera*, AChE's, insensitivity to organophosphates seems to be controlled by a single, autosomal gene Individuals are one of three genotypes (SS, RS. RR) (Fig. 52). Organophosphate insensitive AChE is partially dominant and heterozygotes are much more susceptible to high doses of insecticide than homozygotes .

Methyl paraoxon insensitive AChE, in Australian *H. armigera*, appears to have a real fitness deficit, resulting in slower larval growth. It is also notable, that despite greatly expanded use of organophosphates *H. armigera* on cotton, in Australia, organophosphate resistance frequency remains low overall. This may be a consequence of the lack of fitness in individuals carrying insensitive AChE. There is also a noticeable lack of homozygous resistant (RR) individuals in field populations and it may well may carry a lethal mutation. Our studies are continuing.

f) Rapid biochemical resistance detection techniques.

Introduction

An effective resistance management strategy needs to be supported by rapid and accurate resistance monitoring techniques. In Australia, the performance of the *Helicoverpa* resistance management strategy has largely been monitored by conventional discriminating dose bioassay techniques . However, there are many drawbacks to using bioassay techniques with *H. armigera* because it is slow, labour intensive, expensive and sometimes unreliable. Furthermore, discriminating dose bioassay cannot distinguish between *H. armigera*, which are

heterozygous or homozygous for resistance or even estimate the resistance factor, both of which can have important implications for field control of *H. armigera*.

Resistance mechanism studies have shown that pyrethroid, carbamate and organophosphate in Australian *H. armigera* resistance are as a result of biochemical resistance mechanisms. Pyrethroid resistance in Australian *H. armigera* is caused by a massive overproduction of esterase enzymes which detoxify pyrethroids by sequestration and hydrolysis. Esterase titre is correlated to resistance factor. Carbamate and organophosphate resistance in *H. armigera* are as a result of two different types of insensitive acetylcholine esterase (AChE). A result of resistance mechanism studies, has been the development of rapid biochemical methods for the detection of pyrethroid, carbamate resistance in Australian *H. armigera*. The methods are based on assays for resistance enzymes. Such methods can be utilised for laboratory based resistance detection, and for field based, resistance detection kits.

Methods

Insects and Bioassay

H. armigera eggs were collected from cotton in NSW and Queensland, during the summers of as part of an *H. armigera* pyrethroid and carbamate resistance monitoring programme. In the laboratory, the eggs were reared to larvae, as previously described. Larval bioassays, and biochemical assays were used to determine pyrethroid and carbamate resistance status in the test insects.

Biochemical assays for insecticide resistance.

H. armigera larvae (3-4 mg), were assayed for pyrethroid and/or carbamate resistance using biochemical assays for total esterase activity or AChE insensitivity respectively. Individual 3- 4 mg resistant and susceptible *H. armigera* were homogenised in a microtitre plate in 50 ml of 0.02M phosphate buffer (pH 7.0) containing 0.5% Triton X-100.

Esterase activity was detected with 1-naphthyl acetate as a substrate, using kinetic assays. Aliquots of homogenate (10 ml), were transferred to a clean microplate containing 240 ml of 0.2M phosphate buffer (pH 6.0) with 0.6% Fast Blue RR Salt and 1.86% 1-naphthyl acetate. Kinetic assays were immediately performed on a Bio-Rad 3550 microplate reader (Bio-Rad Laboratories, utilising Kinetic Collector 2.0 software, run on a Macintosh SE microcomputer) taking absorbance readings (450 nm) automatically at 14 second intervals for 10 minutes. Linear regressions were performed by the microcomputer to give kinetic velocities, using an absorbance limit of 2000 mO.D. According to esterase activity levels, individual *H. armigera* were assessed for pyrethroid resistance status, including resistance factor.

Activity of AChE in *H. armigera* was measured by the method of Ellman in which the hydrolysis of the substrate analogue acetylthiocholine iodide (ATChI)is measured colorimetically by the absorbance of 2-nitro - 5- thiobenzoate at 405 nm, after the reaction of 5,5'- dithiobis (2-nitrobenzoate) (DTNB) with the liberated thiocholine. Assays were done in a microplate.

AChE response to carbamate and organophosphate inhibition in *Helicoverpa* larvac was measured as follows. Two 12.5 µl aliquots of each larval homogenate, were transferred to a clean microplate. 100 µl of DTNB was added to each well followed by 100 µl ATChI or ATChI containing a diagnostic insecticide dose of insecticide, ATCh I and DTNB solutions (in 0.01M phosphate buffer, p H 7.5, containing 0.05 % Tritin X-100), gave final concentrations of 0.5 and 0.05 mM respectively. Insecticides chosen for resistance monitoring were methomyl, 110µ M, and methyl para-oxon 33 µM, which resulted in 100% inhibition of enzyme activity in susceptible *H. armigera*. Activity was measured continuously in a Bio-Rad microplate reader for 20 mins, utilising Kinetic Collector software to fit linear regressions. Activity was found to be linear up to an absorbance limit of 50 m OD. Wells containing 100µl of ATChI in buffer served as uninhibited controls. Enzyme activity in the presence of insecticides was calculated as a percentage of the corresponding uninhibited rate. AChE activity in the presence of the discriminating dose of methomyl and methyl paraoxon was used to discriminate between susceptible , heterozygous and homozygous resistant *H. armigera* .

Results

Pyrethroid resistance frequencies (Macquarie Valley, 1993 - 1996), determined by conventional discriminating dose bioassay, are shown in Figure 57. Resistance frequencies obtained by biochemical methods, are insignificantly different (P = 0.05), from those by conventional bioassay. The pyrethroid resistance factor, estimated by biochemical assay, is also shown in Fig 57.

Thiodicarb resistance frequencies, (1992 - 1996) in NSW and Qld cotton areas, determined by conventional bioassay and biochemical methods, are shown in Fig. 58 Results of the biochemical detection of carbamate resistance, are not significantly different (P = 0.05) to data from conventional bioassay methods. The biochemical assay also enabled discrimination between individuals heterozygous and homozygous for resistance.

Discussion

These studies indicate that biochemical assays are an excellent way to monitor pyrethroid and carbamate resistance mechanisms in *H. armigera*. Pyrethroid and carbamate resistance data, which were generated by biochemical assays correlate closely with that obtained by conventional bioassay. In addition, the biochemical assays were able to distinguish the detailed pyrethroid and carbamate resistance status of each insect.

The need for insecticide resistance monitoring programmes within the context of resistance management strategies is well understood. Discriminating dose bioassay methods can be uninformative, slow, labour intensive, expensive and are critically dependent on the LD99 estimate. Biochemical assays for resistance, on the other hand, are rapid, performed on very small larvae and results can be available within minutes. When there is unquestionable evidence that the biochemical processes being measured, are involved in resistance, then we have an excellent means of resistance detection, yielding far more information than discriminating dose bioassay. In the case of *H. armigera*, the biochemical mechanisms of resistance to pyrethroid, carbamate and organophosphate resistance are well understood. Esterase isoenzymes sequester and metabolise pyrethroids, and quantitative changes in enzyme titre are correlated to resistance factor. AChE activity assays measure directly the interaction between the carbamate insecticides and the target protein.

In Australia, rapid and simple biochemical assays show an enormous potential to streamline the spray decision system against *H. armigera*. Since the biochemical assays produce a coloured reaction, it has been possible for us to construct robust, field based resistance detection kits (Figs. 59 - 63). The kits involve a simple egg or larval homogenisation procedure, followed by the addition of substrate and dye and in the case of an assay for insensitive acetylcholinesterase, a carbamate or organophosphate inhibitor. The results can be read accurately by eye after 10 - 20 minutes (Fig 61) and give an indication of resistance frequency, resistance factor and the proportions of individuals heterozygous and homozygous for carbamate resistance. Both pyrethroid and carbamate resistance detection kits have been tested by cotton industry personnel over several years in Queensland and NSW and the information generated correlated excellently to laboratory bioassays. The kits successfully were used to predict insecticide efficacy. At the same time, however, it is essential to be vigilant in monitoring for the appearance of new mechanisms of resistance.

g) New control chemicals for *Helicoverpa*

Introduction

The efficacy of new insecticides which have a potential to control *Helicoverpa* have been investigated in this project. Such chemicals are chlorfenapyr (Cyanamid), the spinosad, Tracer (Dow), amitraz and indoxacarb (Du Pont). While, none of these compounds are very intrinsically toxic to *Helicoverpa*, they do provide adequate control. It is essential that the use of these chemicals be managed to prevent, or delay the development of resistance. Baseline data, against which further changes can be measured, is being accumulated as part of the regular resistance monitoring programme.

Results

The documentation of baseline data for the newer insecticides is very important because *H. armigera* appear to have an almost infinite biochemical capacity to develop insecticide resistance. Already there are indications of resistance to chlorfenapyr and we have documented an esterase mediated resistance mechanism (Fig 64). Chlorfenapyr is a new class of compound, thought to be unaffected by current *H. armigera* resistance mechanisms.

h) Pyrethroid synergism - biochemical studies

(i) Piperonyl butoxide

Pyrethroid resistance in *H. armigera* is largely due to overproduction of specific esterase isoenzymes which sequester and hydrolyse pyrethroids. Piperonyl butoxide (PBO) is partially effective as a pyrethroid synergist in *H. armigera* and its synergistic properties have been used in tank mixes to improve pyrethroid efficacy in the field. Pbo is known to facilitate pyrethroid penetration through the cuticle of resistant *H. armigera* and acts as an inhibitor of pyrethroid resistance related esterases at concentrations of approximately 10^{-5} M (Fig 65). There is no evidence of a monooxygenase mediated resistance mechanism.

The data, which shows that PBO binds to *H. armigera* resistance related esterases, may go a long way toward explaining the partial synergism of pyrethroids by PBO. Up to 70 % of the pyrethroid resistance related esterase activity are apparently inhibited in the presence of PBO. The concentration of PBO needed is quite large (~10^{-5}M), but then, very large doses of PBO are also required to synergise fenvalerate *in vivo*, (10 - 50 µg/larva) so internal concentrations of Pbo in *H. armigera* larvae could also be very large.

PBO has been assumed to inhibit monooxygenase-based detoxification of pyrethroids in resistant *H. armigera* although, there is no indication of cytochrome P450 mediated metabolism of pyrethroids. Instead there is compelling evidence to support an esterase mediated metabolic resistance mechanism..

Our data show that Pbo only partially inhibited *H. armigera* resistance associated esterases and this is consistent with bioassay data. Survival of Pbo/fenvalerate is restricted to more highly resistant individuals (>30 fold) because PBO does not have a limitless capacity to inhibit esterase. Highly resistant *H. armigera* which can have enormous quantities of esterase, probably more than enough to detoxify pyrethroids even after some binding with Pbo has occurred.

Pbo has been used as a tank additive to pyrethroid sprays against *H. armigera* in Australia but our data shows that Pbo should be used in the field with great caution, as it will select for more highly resistant individuals and so lead to control failures.

(ii) Propargite (Comite®)

Propargite, or Comite® is considered to have PBO like, pyrethroid synergism properties. 1 To test this hypothesis, propargite was incubated with resistant *H. armigera* homogenates and the effects of propargite on esterase activity were monitored. Results showed that propargite is a moderately effective esterase inhibitor, apparently acting in the same way as PBO and binding to *H. armigera* esterase (Fig. 66).

(iii) Organophosphates as esterase inhibitors and pyrethroid synergists

Introduction

Biochemical studies show that pyrethroid resistance associated esterases in *H. armigera* are inhibited by organophosphorous compounds. Esterase inhibition by organophosphates does not occur immediately after dosage, but occurs rapidly, with maximum enzyme inhibition from 2 to 24h after dosage, depending on the inhibitor used. Enzyme inhibition studies are supported by pyrethroid bioassay using organophosphate synergists, showing excellent levels of pyrethroid synergism by organophosphates against resistant *H. armigera*. Up to hundred percent mortality could be achieved. Use of organophosphate synergists in the field may have the potential to restore pyrethroid susceptibility in Australian *H. armigera*.

Results

Inhibition of esterase activity by organophosphates

Gel electrophoresis and assays of total esterase activity indicated that all organophosphates used in these experiments bound to *H. armigera* esterases, and in particular, those bands implicated in pyrethroid resistance, (Figs. 67, 68).

Acephate rapidly bound to *H. armigera* esterases, (0.5 - 1h. after treatment), however this effect was short lived and esterase activity in larvae, was completely recovered 8h after treatment. Chlorpyrifos and its oxon also rapidly inhibited *H. armigera* esterases, however, recovery of activity was slow, with full enzyme activity not recovered until 36 h after treatment. Inhibition of esterase activity by profenofos occurred primarily between 4 and 8 h after treatment (at 8 h, some 70% of esterase activity had been inhibited) . However, recovery of enzyme activity was rapid. By contrast, ethion caused almost complete inhibition of all esterase activity, between 1 to 72 h. after application.

Organophosphate /Pyrethroid Synergism

All organophosphate used, synergised pyrethroids during the time of maximum enzyme inhibition, however, the greatest effects were obtained with the less resisted pyrethroids (Figs 69, 70). Pre-treatment with ethion, greatly increased pyrethroid mortality, and the increased period of susceptibility persisted to 24 hours after pre-treatment with synergist, synergistic effect declined over longer time intervals (Fig 69). Acephate (Fig 69), also showed a strong, but short lived synergistic effect with pyrethroids , These effects rapidly declined with time intervals, greater than 3h between acephate and pyrethroid applications. Profenofos (Fig 70), was also synergistic with pyrethroids. However, these effects were not rapid, a 4h time interval between application of profenofos and pyrethroid was required to reach maximum mortality and the effects rapidly declined thereafter. Chlorpyrifos (Fig 70) was also shown to be an reasonably effective pyrethroid synergist. Maximum mortality, was achieved after a 4 h, pre-treatment time, however, this level of mortality rapidly decreased.

Discussion

These data showed that organophosphate inhibition of resistant *H. armigera* esterase resulted in effective pyrethroid synergism, especially with the more effective, less resisted, pyrethroids. Ethion (which is non-toxic and therefore could be applied in larger doses) was a more effective synergist than any other and was capable of producing 100% mortality.

These results open up the possibility that organophosphate synergists could be used in Australia to restore at least some pyrethroid susceptibility in *H. armigera*. The potential use of organophosphates as pyrethroid synergists would need to be carefully incorporated into a resistance management strategy because uncontrolled use of synergists designed to eliminate a major metabolic pyrethroid resistance mechanism may well result in the selection of other resistance mechanisms, such as Kdr.

i) Pyrethroid / esterase binding in *H. armigera*

Introduction

Pyrethroid resistance in Australian *H. armigera* is largely due to overproduction of specific esterase isoenzymes (R_m 0.24 - 0.33), which sequester and hydrolyse pyrethroids. *H. armigera* are resistant to all pyrethroids, however, the resistance factor varies according to pyrethroid used. The objective of this study was to examine the binding of pyrethroids to *H. armigera* esterases and to determine the relationship between esterase binding to pyrethroids and resistance factor.

Results

Bioassay Data

Pyrethroid efficacy against resistant *H. armigera* varied considerably, (Fig. 71). Least effective pyrethroids were flucythrinate (86 x) and fenvalerate (55x) and es-fenvalerate (32 x), while the zeta-cypermethrin, bifenthrin, deltamethrin, permethrin, alpha-cypermethrin resistance factors were only 11 - 15 fold.

Pyrethroid inhibition of esterase activity

Pyrethroids were incubated with resistant *H. armigera* homogenates. All pyrethroids used, inhibited esterase activity in resistant *H. armigera*. (Fig. 72). However, there were considerable differences between pyrethroids. Fenvalerate and flucythrinate resulted in almost complete inhibition of esterase activity. Esterases, however, did not bind as readily to deltamethrin, alpha-cypermethrin, zeta-cypermethrin, permethrin and bifenthrin and even at the highest pyrethroid concentrations, only 30 - 40 % of esterase activity was inhibited. Es-fenvalerate was intermediate in esterase binding ability, between these two pyrethroid groups. The marked differences between the ability of pyrethroids to inhibit *H. armigera* esterase activity is also shown by polyacrylamide gel (Fig 73). Homogenates were incubated with concentrations of fenvalerate and zeta-cypermethrin (0.8 - 8.0 µM). Diminished esterase activity was very pronounced in homogenates incubated with fenvalerate, compared to zeta cypermethrin.

Discussion

Bioassay results indicated that all pyrethroids did not act identically on resistant *H. armigera*,(Fig 70). Flucythrinate, fenvalerate and es-fenvalerate, were the least effective while permethrin, deltamethrin, bifenthrin, zeta-cypermethrin and alpha-cypermethrin were more toxic to resistant *H. armigera*,. Inhibition studies also showed real differences between the ability of pyrethroids to bind to esterase enzymes . Fenvalerate and flucythrinate, which had largest resistance factors, bound very readily to esterases. On the other hand, permethrin, deltamethrin, bifenthrin, Z-cypermethrin and a-cypermethrin, which are less resisted, less readily inhibited esterase activity, even at higher insecticide concentrations, Es-fenvalerate, was intermediate in both resistance factor and esterase inhibitory ability. From these data, it seems therefore likely that pyrethroid resistance factor in *H. armigera* was determined by differing ability of esterase isoenzymes to bind to each pyrethroid.

An examination of the structures of the pyrethroids used in this study, indicates marked differences in structure of the ester-pyrethroids which interacted more readily with *H. armigera* esterases and those which bound less readily. The former group, (fenvalerate, flucythrinate and es-fenvalerate), are pyrethroids which contain a halogenated benzyl group. While in the latter group of pyrethroids (permethrin, deltamethrin, bifenthrin, Z-cypermethrin and a-cypermethrin) the benzyl ring was replaced by a dihalogenated aliphatic entity. How this change in pyrethroid structure would effect esterase mediated detoxification in *H. armigera* is unknown, but will form the subject of future studies. Our findings of esterase/pyrethroid binding, also provide information which can be used to rapidly screen for, or even design new pyrethroids which will be much more effective against resistant *H. armigera*. These biochemical assays are much cheaper, rapid and more informative then conventional bioassay.

j) Bifenthrin resistance in *H. armigera*

Introduction

While esterase isoenzymes which are responsible for resistance to pyrethroids in *H. armigera*, can contribute to bifenthrin resistance (see section 8), until recently most populations of pyrethroid resistant *H. armigera* were effectively susceptible to bifenthrin. However, in recent years, there been a considerable increase in the frequency of *H. armigera* larvae are resistant to bifenthrin.

Methods

Insects

A bifenthrin selected strain was obtained by retaining and rearing *H. armigera* which were survivors of the discriminating dose of bifenthrin (0.1 µg). The moths were combined, mated and the subsequent F_1 and F_2

generation subject to further selection with a doses of bifenthrin 5 x and 10 x the discriminating dose respectively.

An unselected, bifenthrin susceptible, strain of *H. armigera* , which originated from NSW and Queensland *H. armigera* populations was used in this study . The strain was approximately 35 fold x resistant to fenvalerate.

Insecticides and Bioassay
Technical grade bifenthrin was dissolved in acetone and solution prepared by serial dilution. Larvae were bioassayed by topical application as previously described.

Biochemistry
Bifenthrin resistant *H. armigera* homogenates were incubated for 30 mins with bifenthrin). Esterase activity was detected, as described elsewhere in this report . Final concentrations of bifenthrin were 0.01 - 2 μM.

Electrophoresis
Homogenates incubated with bifenthrin were also run on polyacrylamide gels using electrophoresis techniques and stained for esterase activity using 1-naphthyl acetate as a substrate.

Electrophysiology
H. armigera larvae were dissected to expose the peripheral;nerves in the body wall. The insect was flooded with physiological saline. Muscles in the body wall were used to make measurement of spontaneous activity from the peripheral nerves, which were picked up by a suction electrode and the preparation grounded The recording electrode output was connected to a 1000 gain pre-amplifier 1000x and Mac Lab system. Electrical activity of the nerves was displayed on the computer screen. Spontaneous nerve activity was recorded in saline and then replaced with saline containing bifenthrin. 10^{-5} M bifenthrin was the minimum concentration required to induce repetitive firing within 5 mins in the susceptible strain, and was chosen as the diagnostic concentration.

Results and Discussion

Table 9

Toxicity of bifenthrin to resistant ant susceptible populations of *H. armigera* 3rd instar larve.

Strain	LD_{50} μg/larva	Slope	RF*	DF
Susceptible	0.034	4.2	-	1.1
Resistant	0..58	3.4	17.1	1.4

* Resistance factor calcualted as the ration of LD_{50} [R] / LD_{50} [S]

Results of bioassays are shown in Table 9 . The resistance factor of bifenthrin selected strain was approximately 17 fold, compared to the susceptible strain.

Electrophysiological data indicated that in both resistant and susceptible strains, there was a rapid onset (within 25 mins), of nerve firing (Figs 74,75). Bifenthrin, like other pyrethroids causes repetitive firing of the nervous system in the insect, leading to death. Thus, it was evident that bifenthrin resistance in *H. armigera* was not caused by nerve insensitivity to pyrethroids (Kdr).

Biochemical investigation showed, that while bifenthrin typically binds poorly to pyrethroid resistant esterases in *H. armigera*, esterases in specifically bifenthrin resistant *H. armigera* , had a much greater affinity for bifenthrin. Up to 50% of total esterase activity was inhibited in the resistant strain. (Fig. 76). An analysis of esterase banding patterns on polyacryamide gels showed that there appeared to be several bands showing

diminished staining due to esterase / insecticide binding (Fig. 77). In particular, there were some bands in the region RM ~0.5 , which completely disappeared with increasing insecticide concentration .

Discussion

These studies indicated that *H. armigera* has developed a new esterase mediated, bifenthrin resistance mechanism, which is in addition to esterases which confer resistance to other pyrethroids. Bifenthrin resistant *H. armigera* have developed forms of esterase enzymes which are more efficient at dealing with bifenthrin . Preliminary enzyme kinetic studies support the view, that differing forms of the esterases being evolved.

Considering the extreme selection pressure *H. armigera* have received from bifenthrin in recent years, it is hardly surprising that cotton populations have apparently developed more efficient enzymes. Additional bioassay studies indicate that this additional resistance is specific to bifenthrin and work is continuing.

k) Pyrethroid resistance in *H. punctigera*

Introduction

Resistance development in *Helicoverpa* spp. has been linked to a combination of genetic, ecological, behavioural and agronomic factors In Australia, both *H. punctigera* and *H. armigera* have been mainly controlled by insecticides, especially on cotton. It has been assumed that while *H. armigera* has developed high frequencies of resistance to insecticides and *H. punctigera* could not due to significant differences in genetic, ecological or behavioural factors between these species. However, as noted in section 1, resistance to pyrethroids, endosulfan and methomyl have been recorded in *H. punctigera*. This section reports a study of pyrethroid resistance mechanisms in *H. punctigera*.

Methods

Strains and electrophoresis

Fenvalerate susceptible and resistant (17 fold), populations *H. punctigera* (originating from selection of field strains) were used in this study.

Polyacrylamide gel electrophoresis preparation methods and esterase assays have been described elsewhere in this report.

Inhibition of enzyme activity by fenvalerate

Esterase activity in the presence of inhibitors was detected using 1-naphthyl acetate as a substrate, using kinetic assays. Groups of 50 individual (3- 4 mg) resistant and susceptible *H. punctigera were* homogenised in eppendorf tubes in 1.0 ml of 0.02M phosphate buffer (pH 7.0) containing 0.05% Triton X-100. Homogenates were centrifuged for 3 mins, at 750g and decanted into clean eppendorf tubes. Fenvalerate (dissolved in acetone) was added in 1ml of acetone to the *H. punctigera* homogenates at various concentrations, (fenvalerate ,0.1 - 500 mM: profenofos 0.1 - 110 mM). Homogenates were incubated at 25 ^{o}C , as were controls (insect homogenate alone and homogenate plus acetone). At 30 mins after the addition (10 - 180 mins), aliquots (10 ml) were assayed for esterase activity.

In vitro es-fenvalerate metabolism

[14 C] es-fenvalerate (18.4 mCi/mmole), universally labelled in the phenoxybenzyl ring, a to the cyano group, was provided by Sumitomo Chemicals. Radiochemical purity was verified as > 99%. Three potential metabolites (3-phenoxybenzyl alcohol, 3-phenoxybenzaldehde and 3-phenoxybenzoic acid) which have been detected as metabolites of fenvalerate were purchased as analytical standards.

To investigate the metabolism of es-fenvalerate in resistant and susceptible *H. punctigera* the following methods were employed. Twenty 3rd instar larvae (30 - 40 mg) per replicate were homogenised in a centrifuge tube in 2 ml of 0.02M phosphate buffer, (pH. 7.0). Tubes were centrifuged at 750g for 5 mins. The supernatant was divided in two and decanted into clean tubes. The homogenates were either: (1) , incubated at 25°C with 0.25 mg (6000 dpm)^{14}C es-fenvalerate, or (2), incubated with profenofos (at concentration of 10^{-5}M) for 30 mins then further incubated after the addition of 0.25 mg (6000 dpm)^{14}C fenvalerate. Ninety, 150 or 240 mins after the addition of fenvalerate , samples (100 ml) were taken and the reaction stopped by the addition of 200 ml of very cold acetone (- 20°C). Each sample was "spotted" onto a TLC plate and chromatographed alongside ^{14}C fenvalerate and common metabolites. Experiments were replicated 3 times. The mean ^{14}C recoveries were calculated as a percentage of the applied ^{14}C. The data were analysed by ANOVA with Tukeys test for mean separation.

All TLC plates were chromatographed in toluene (saturated with formic acid): ether (5:1 by volume), and the es-fenvalerate and metabolite products were located under ultraviolet light or by autoradiography. Labelled compounds were quantified by gently scraping relevant areas of the TLC plates and suspending the fine powder in 1 ml distilled water and 9-ml emulsifier scintillator for counting radioactivity.

Results

The results of the electrophoretic examination of fenvalerate resistant and susceptible *H. armigera* are presented in Fig 78. Gels of susceptible *H. punctigera*, stained for esterase isoenzyme activity showed two major esterase activity bands, at electrophoretic mobility 0.34 and 0.55, although in some individuals the band at 0.55 was not clearly defined. As well, there were some other very indistinct bands. Fenvalerate resistant *H. punctigera* , possessing all the above bands, had additional densely staining bands in the region R_m, 0.24 - 0.55 and at Rm 0.61. These data suggest esterase mediated detoxification.

The results of *in vitro* metabolism of fenvalerate experiments in resistant and susceptible *H. punctigera*, using [14 C] labelled es-fenvalerate are in Table 10. There was no evidence of fenvalerate metabolism in the susceptible strain but considerable quantities of metabolites were recovered from resistant homogenates during 4 hours incubation and earlier. At 2.5 and 4 h, significant quantities of 3-phenoxybenzoic acid were recovered and traces of 3-phenoxybenzylalcohol. The metabolites recovered, are consistent with enhanced hydrolytic detoxification of fenvalerate by resistant *H. punctigera*.

Studies of esterase activity in the presence of fenvalerate (30 min incubation), is shown in Fig. 79. While fenvalerate had no inhibitory effect on susceptible enzyme activity, in the resistant strain, concentrations of greater than 0.1 mM (30 mins incubation) inhibited the hydrolysis of 1-naphthyl acetate indicating that fenvalerate had bound to the resistant esterase isoenzymes. At concentrations of 2.5 mM and greater, approximately 70 % of enzyme activity was inhibited. Clear inhibition of esterase activity by fenvalerate in resistant *H. punctigera* is also shown by gel (Fig 79).

Discussion

Biochemical studies indicated that pyrethroid resistant *H. punctigera*, had a greatly increased esterase activity toward 1-naphtbyl acetate compared to susceptibles, also showing bands that were not present in the susceptible strain. *In vitro* metabolism studies produced good yields of metabolites which were consistent with ester cleavage of fenvalerate. Enzyme activity studies further showed that resistant *H. punctigera* esterases bound strongly to fenvalerate and it is also possible that further fenvalerate detoxification occurred via esterase sequestration of fenvalerate. *Helicoverpa* insecticide resistance in Australia, has been the subject of a management strategy in northern NSW and southern and central Queensland since 1984. The strategy was primarily designed to contain insecticide resistant *H. armigera*, as it was assumed that ecological factors alone, were sufficient to control the development of field resistance in *H. punctigera*. However, the present data show that, these assumptions are questionable, and that *H. punctigera* can develop field resistance to insecticides. Therefore, caution should be exercised, to limit the exposure of *H. punctigera* to all insecticides.

Table 10

In vitro metabolism of 14-C fenvalerate (labelled in the phenoxybenzyl ring) by homogenates of resistant and susceptible 3rd instar *Helicoverpa punctigera* larvae[1].

TLC isolates	R*f*	1.5 h	2.5h	4h
Susceptible				
es-fenvalerate	0.94	91[a]	95[a]	93[a]
3-phenoxybenzaldehyde	0.87	-	-	-
3-phenoxybenzylalcohol	0.45	-	-	-
3-phenoxybenzoic acid	0.33	-	-	-
lost 14-C		9[d]	5[d]	7[d]
Resistant				
es-fenvalerate	0.94	89[a]	71[b]	62[b]
3-phenoxybenzaldehyde	0.87	-	-	-
Unknown 1	0.66	-	-	-
3-phenoxybenzylalcohol	0.45	-	5[d]	15[c]
3-phenoxybenzoic acid	0.33	7[d]	20[c]	24[c]
Lost 14-C		4[d]	4[d]	-

[1] Results are expressed as the percentage of applied ^{14}C and indicate the mean value of three replicates. Results followed by the same letter are not significantly different (P = 0.05).

6. GENERAL CONCLUSIONS

Research from this project has exceeded the expected outcomes, compared to project objectives. As well as providing a comprehensive resistance monitoring programme, the project has resulted in a good understanding of the mechanisms of resistance to the older chemical groups (pyrethroids, carbamates and organophosphates) in both *H. armigera* and*H. punctigera*. There are excellent prospects of overcoming resistance to such compounds as pyrethroids in the field by use of appropriate synergists and the design of resistance breaking pyrethroids.

7. IMPACT OF RESEARCH PROJECT FOR COTTON INDUSTRY

Insecticide resistance in *Helicoverpa* spp has long been and continues to be, a major threat to the economic production of cotton in Australia. Due to the variable performance of transgenic cotton against *Helicoverpa* spp., insecticides will remain an important component of integrated pest management practices on cotton. This project has provided the data which underpins the *Helicoverpa* resistance management strategy, both in resistance monitoring data and, in information on resistance mechanisms and genetics of insecticide resistance. The management of resistance can only be achieved by accurate resistance monitoring and a clear understanding of resistance mechanisms. Effective resistance management will enable the Australian cotton industry to continue to grow cotton.

Another major benefit from this project has been the development of biochemical methods for the detection of resistance in *H. armigera*. Conventional bioassay is expensive, time consuming and results may not be available for week s after sample collection. The use of rapid, inexpensive, biochemical resistance detection methods, have the capacity to greatly cut the costs of resistance monitoring. The methods can also be used for field based resistance detection and ensure that results have some field relevance.

8. PROJECT TECHNOLOGY

A major benefit of biochemical resistance mechanism studies and resistance detection methods, has been the development of resistance detection kits for pyrethroid, carbamate and organophosphate resistance for *Helicoverpa* spp. These kits can successfully diagnose resistance in the field and can be used by non-expert operators. The kits have been trialed by cotton industry personnel and have proved popular and useful in assisting users to make improved spray decisions.

9. RECOMMENDATIONS

- That research monitoring insecticide resistance and the mechanisms of insecticide in *Helicoverpa* spp. be maintained.

- One of the important roles in dissemination of resistance monitoring data has been the practice of giving each cotton growing area, comprehensive data about the resistance status in *Helicoverpa* spp in that area. This information is vital for predict ing the efficacy of insecticides in the field and industry personnel must be capable of absorbing and using the information. It is recommended that we continue to increase the knowledge base of cotton industry personnel

- It is recommended that steps be taken to commercialise the resistance detection kits. There is a great need for such technology and people in the cotton industry are asking that the kits be made generally available. As it is, we are unable to supply the demand.

10. PUBLICATIONS ARISING FROM RESEARCH PROJECT

Gunning, R. V. , Moores, G. D. and Devonshire, A. L. (1996) - Esterases and fenvalerate resistance in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology* 54: 12 - 23.

Gunning, R. V. , Moores, G. D. and Devonshire, A. L. (1996) - Insensitive acetylcholine esterase and resistance to thiodicarb in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology,* 55: 21 - 28.

Gunning, R.V. (1996) - Bioassay for detecting pyrethroid nerve insensitivity in Australian *Helicoverpa armigera*. *Journal of Economic Entomology*, 89: 816 -819.

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Gunning, R. V., Moores, G. D. and Devonshire, A. L. (1997) - Esterases and fenvalerate resistance in field populations of *Helicoverpa punctigera* Hübner (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology,* **58**: 155 - 162.

Gunning, R. V., Moores, G. D. and Devonshire, A. L. (1997) - Biochemical resistance detection in Australian *Helicoverpa armigera* In *Recent Research Developments in Entomology*, Research Signpost, 1: 203 - 213.

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Gunning, R. V. , Moores, G. D. and Devonshire,A. L., - Esterase inhibitors restore pyrethroid susceptibility in Australian *Helicoverpa armigera, Proceedings of World Cotton Conference 2, Athens, 1998*, (in press).

Gunning, R. V. , Moores, G. D. and Devonshire, A. L. - Biochemical resistance detection: A tool for resistance management in *Helicoverpa armigera. Proceedings of Regional Consultation on Insecticide Resistance Management in Cotton*, Multan, Pakistan, June 1999 (in press).

11. ACKNOWLEDGEMENTS

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Fig. 1

Helicoverpa armigera abundance in the Macquarie Valley

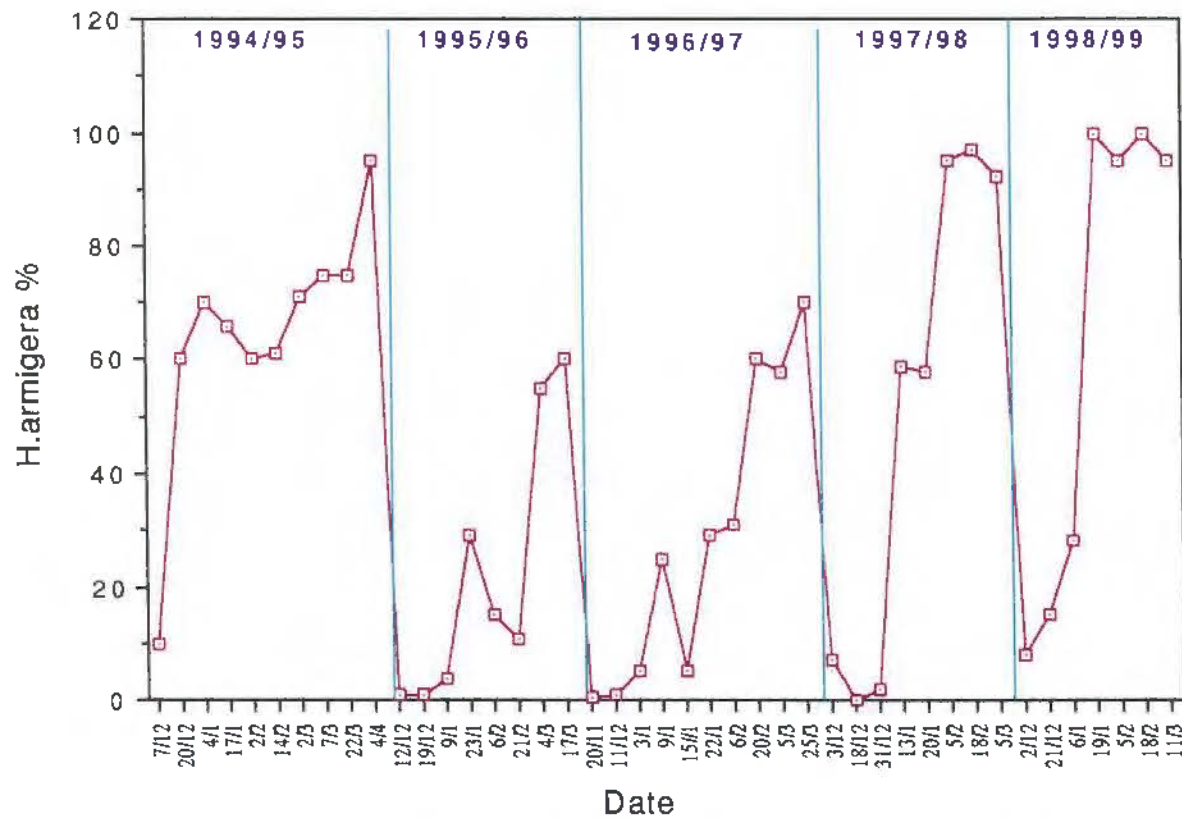


Fig. 2

Macquarie Valley - fenvalerate

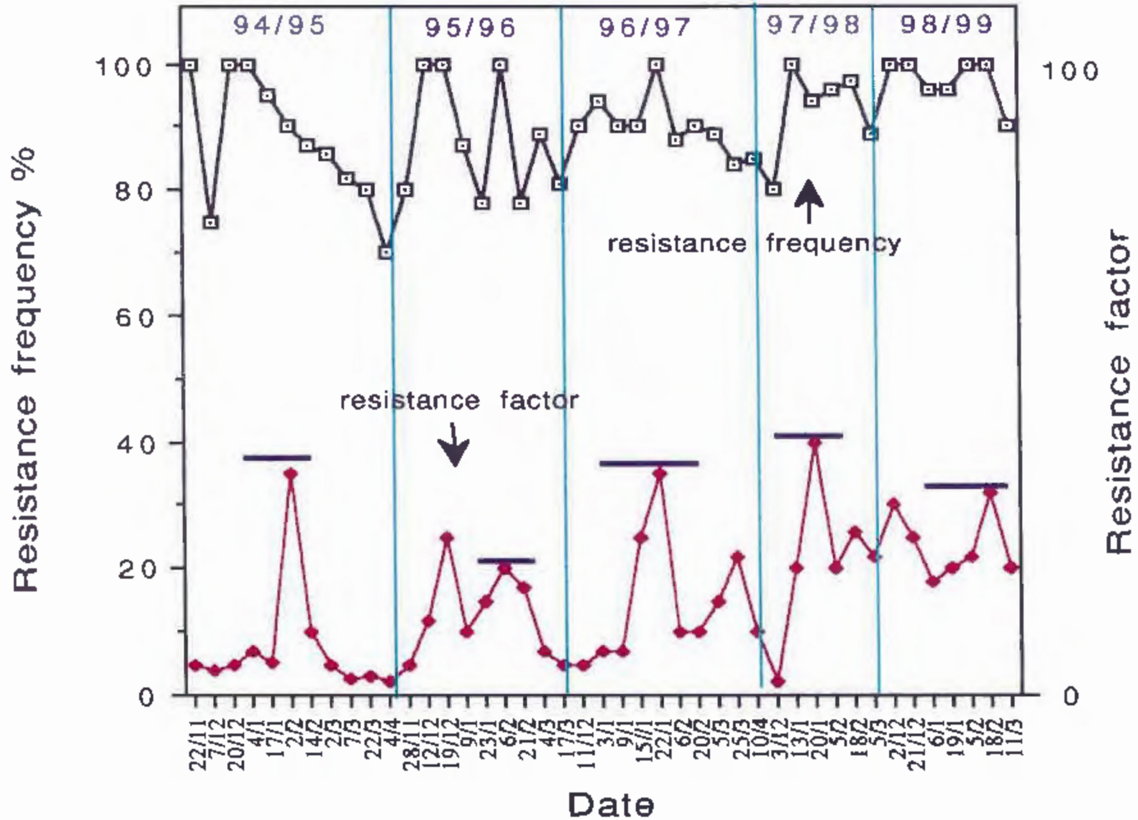


Fig. 3

Macquarie Valley - bifenthrin

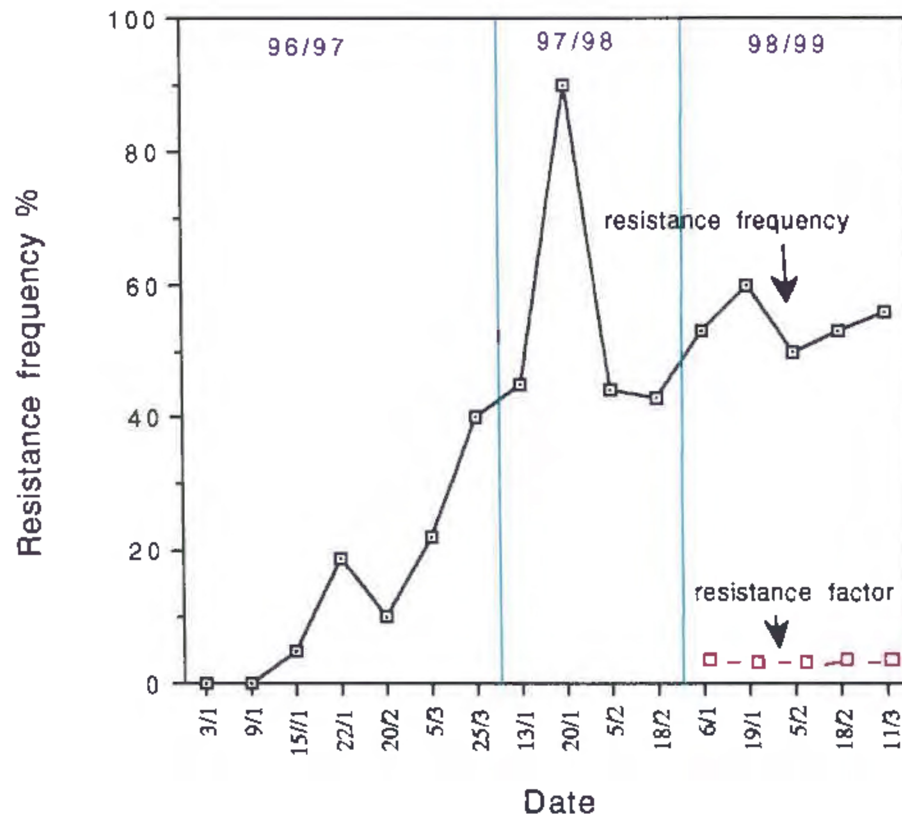


Fig. 4

Macquarie Valley - carbamates

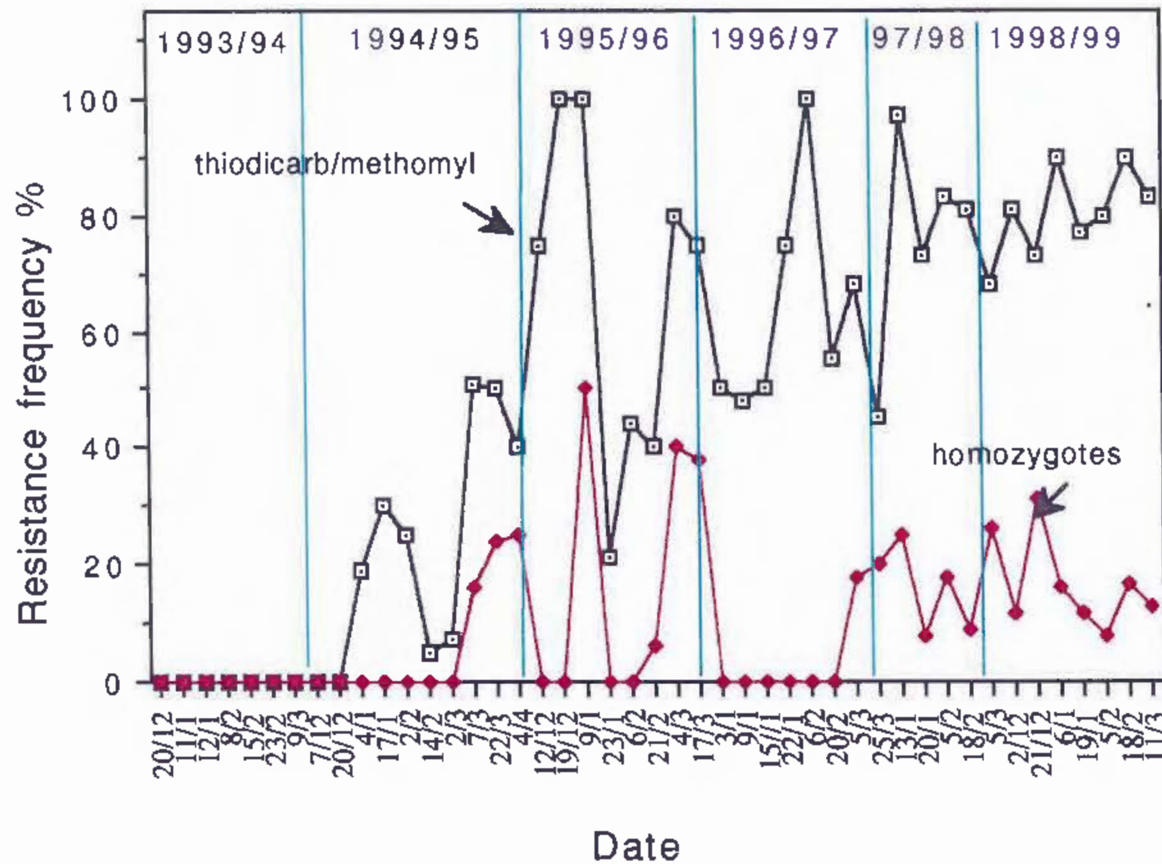


Fig. 5

Macquarie Valley - organophosphates

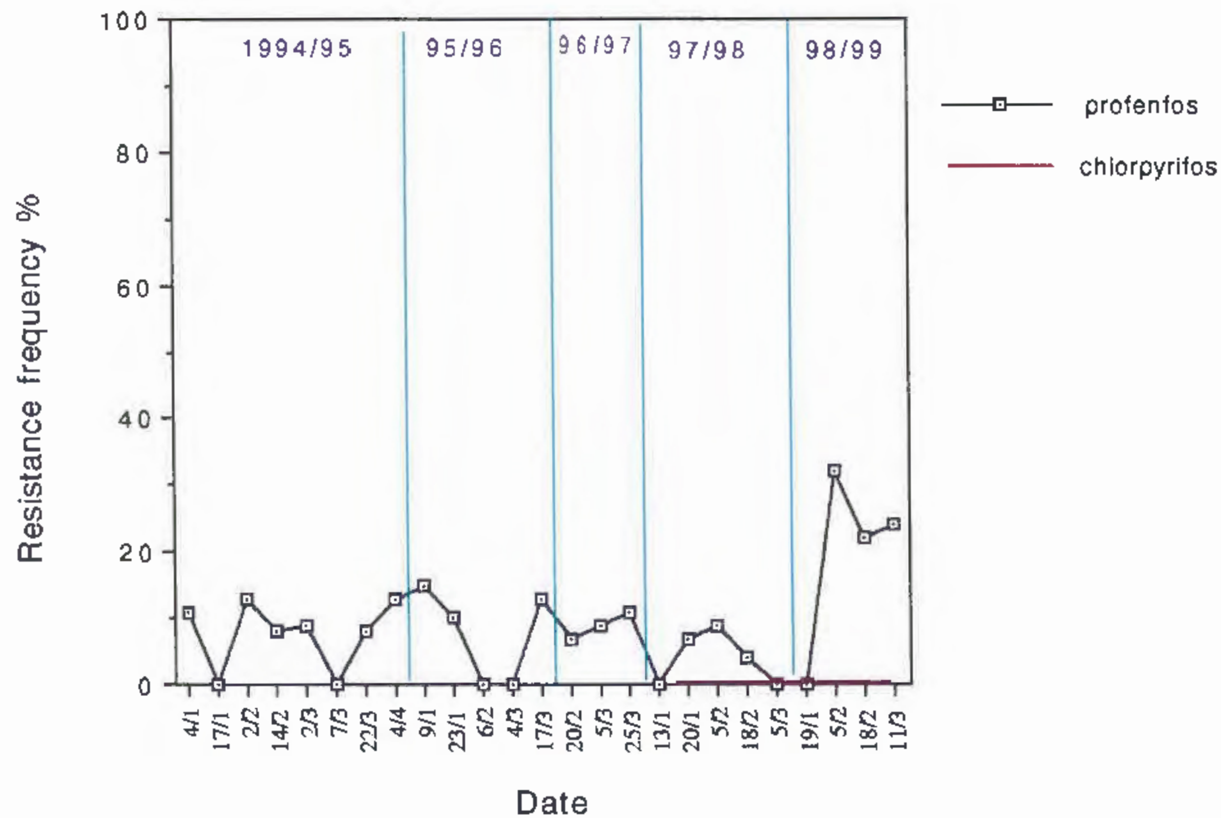


Fig. 6

Macquarie Valley - endosulfan

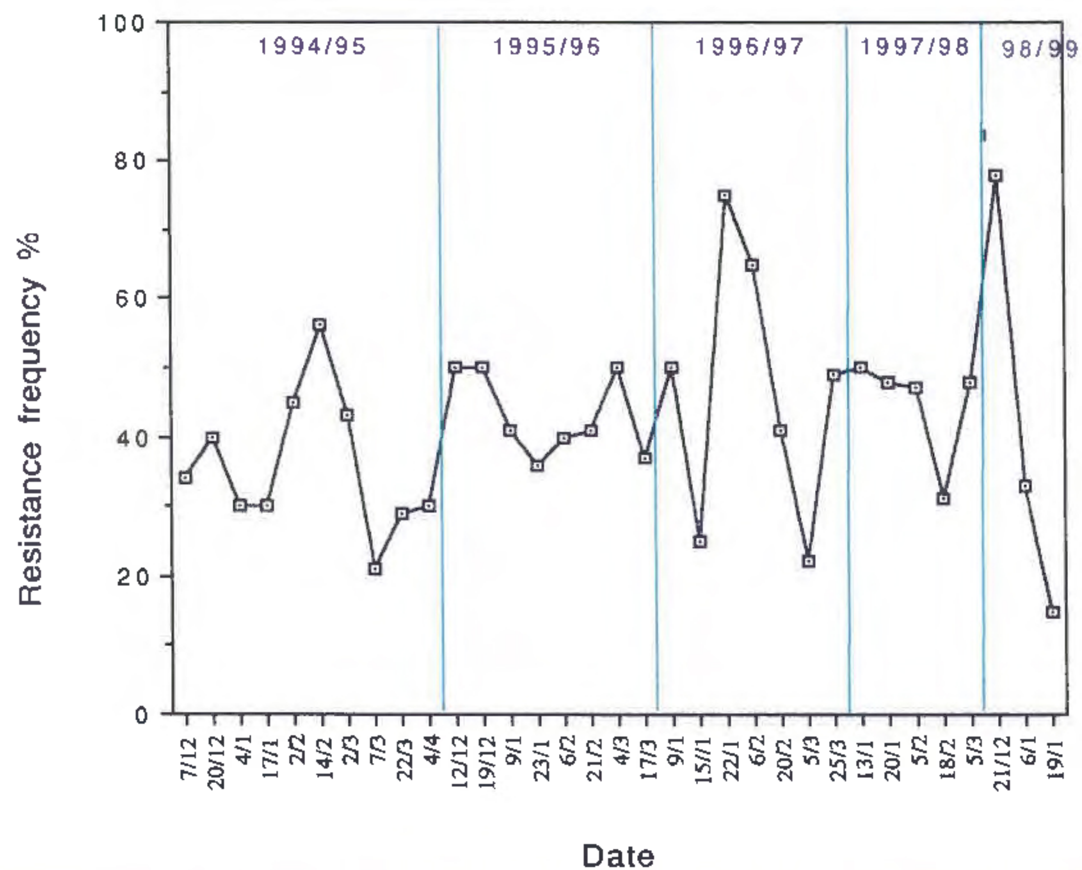


Fig. 7

Upper Namoi - fenvalerate

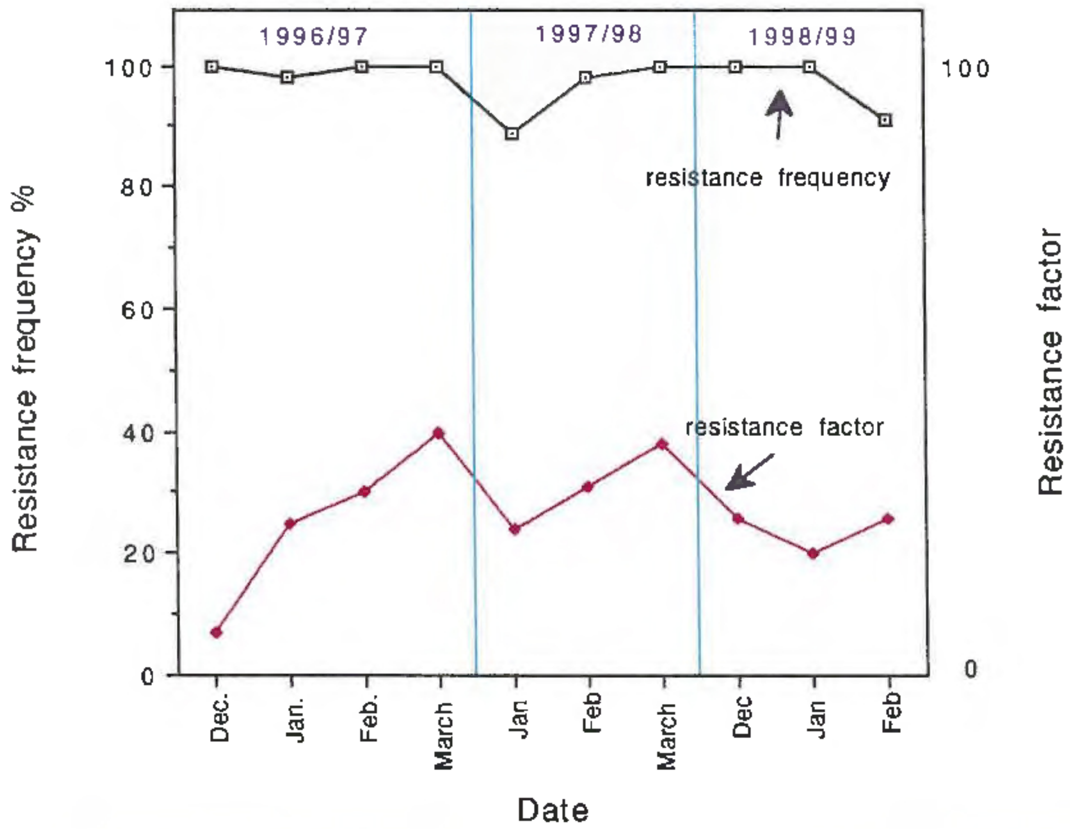


Fig. 8

Upper Namoi - carbamates

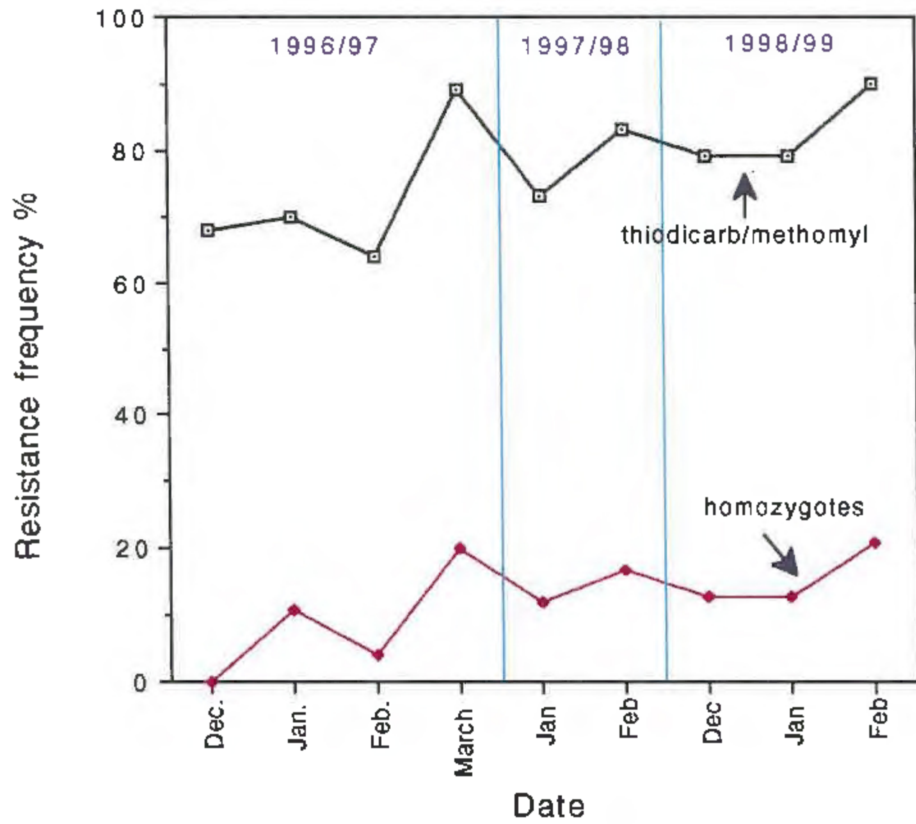


Fig. 9

Upper Namoi - organophosphates

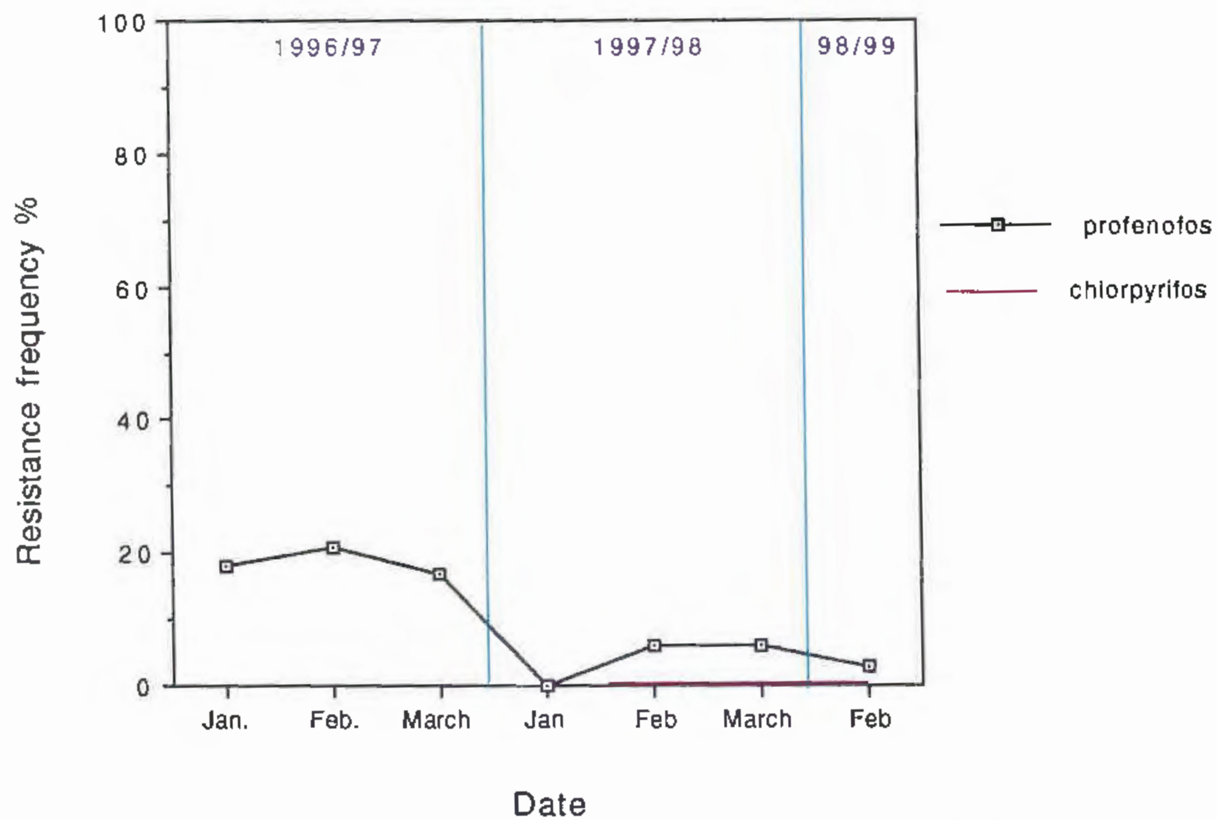


Fig. 10

Lower Namoi - pyrethroids

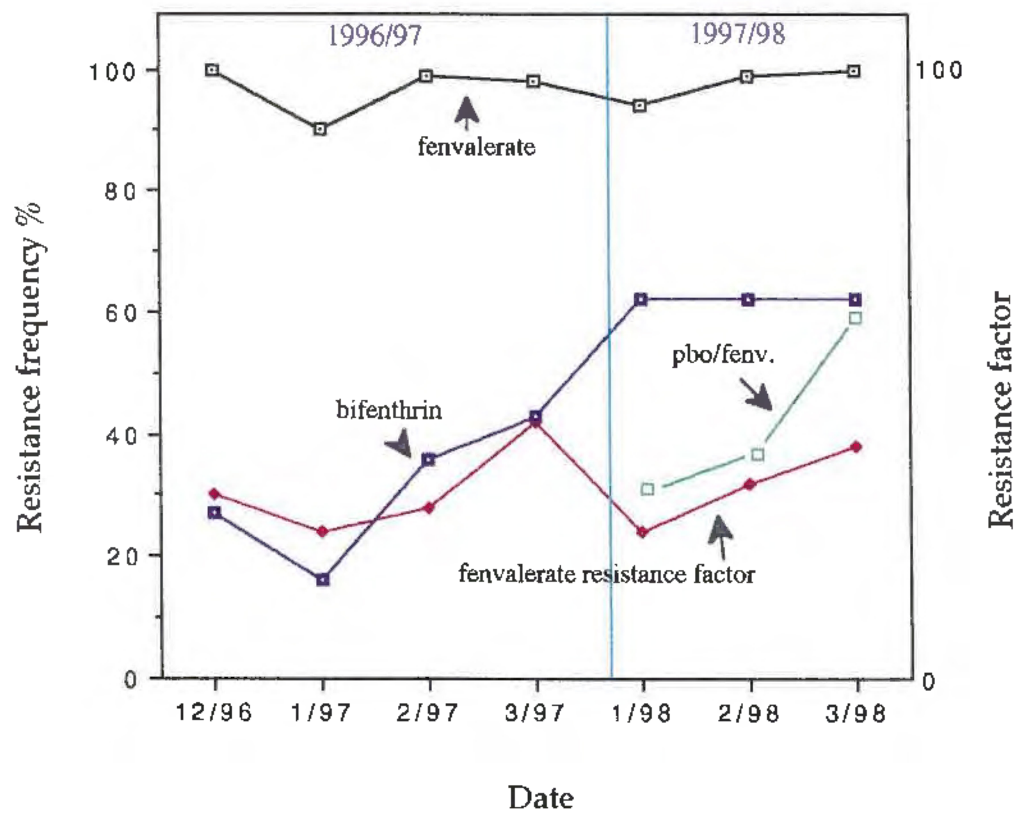


Fig. 11

Lower Namoi - carbamates

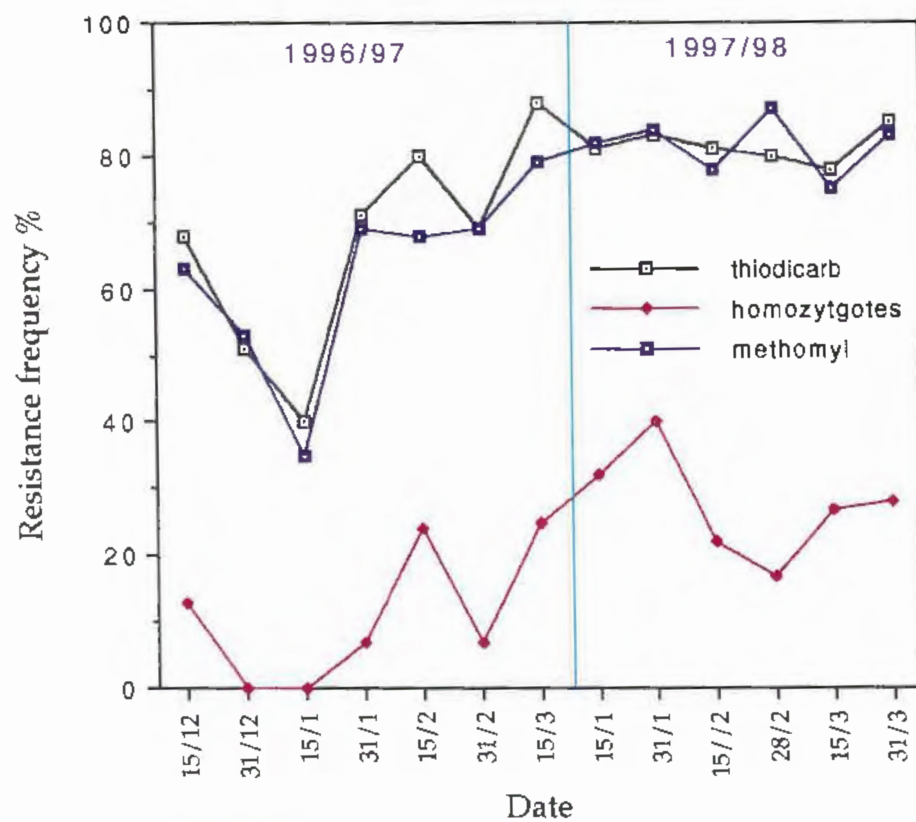


Fig. 12

Lower Namoi - organophosphates

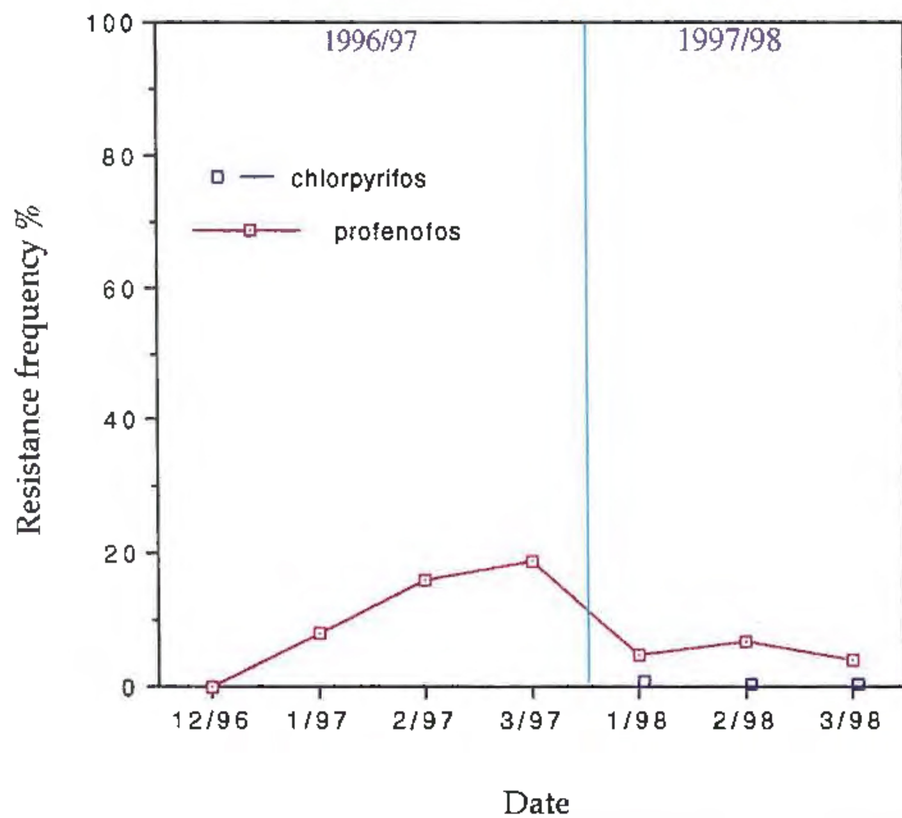


Fig. 13

Lower Namoi - endosulfan

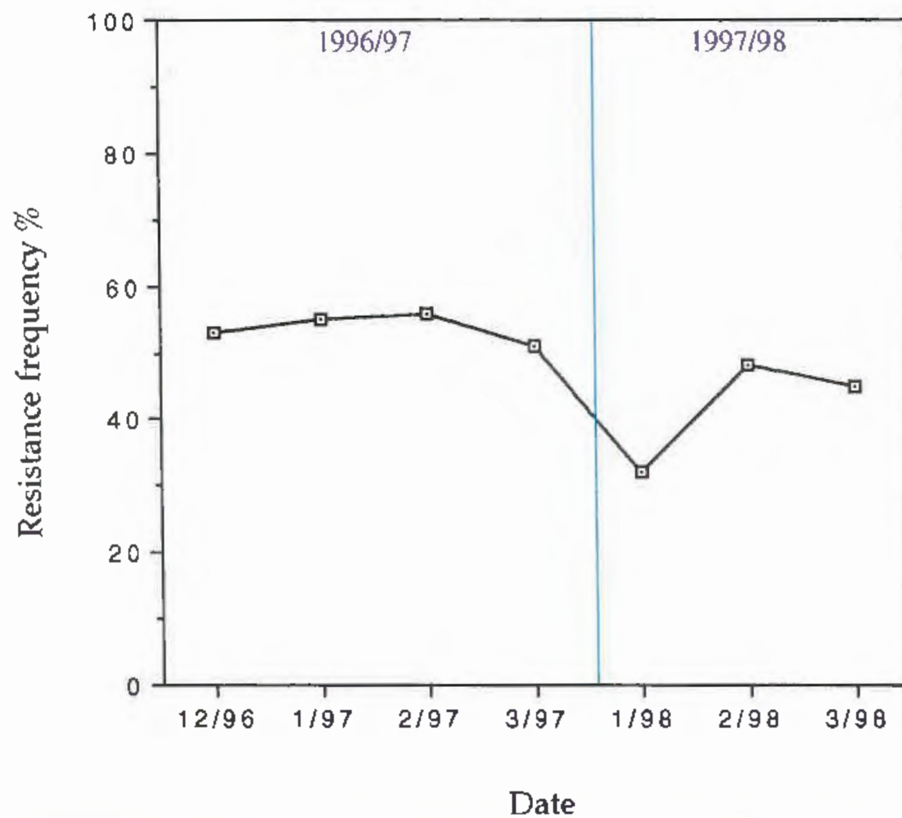


Fig. 14

Gwydir valley - fenvalerate

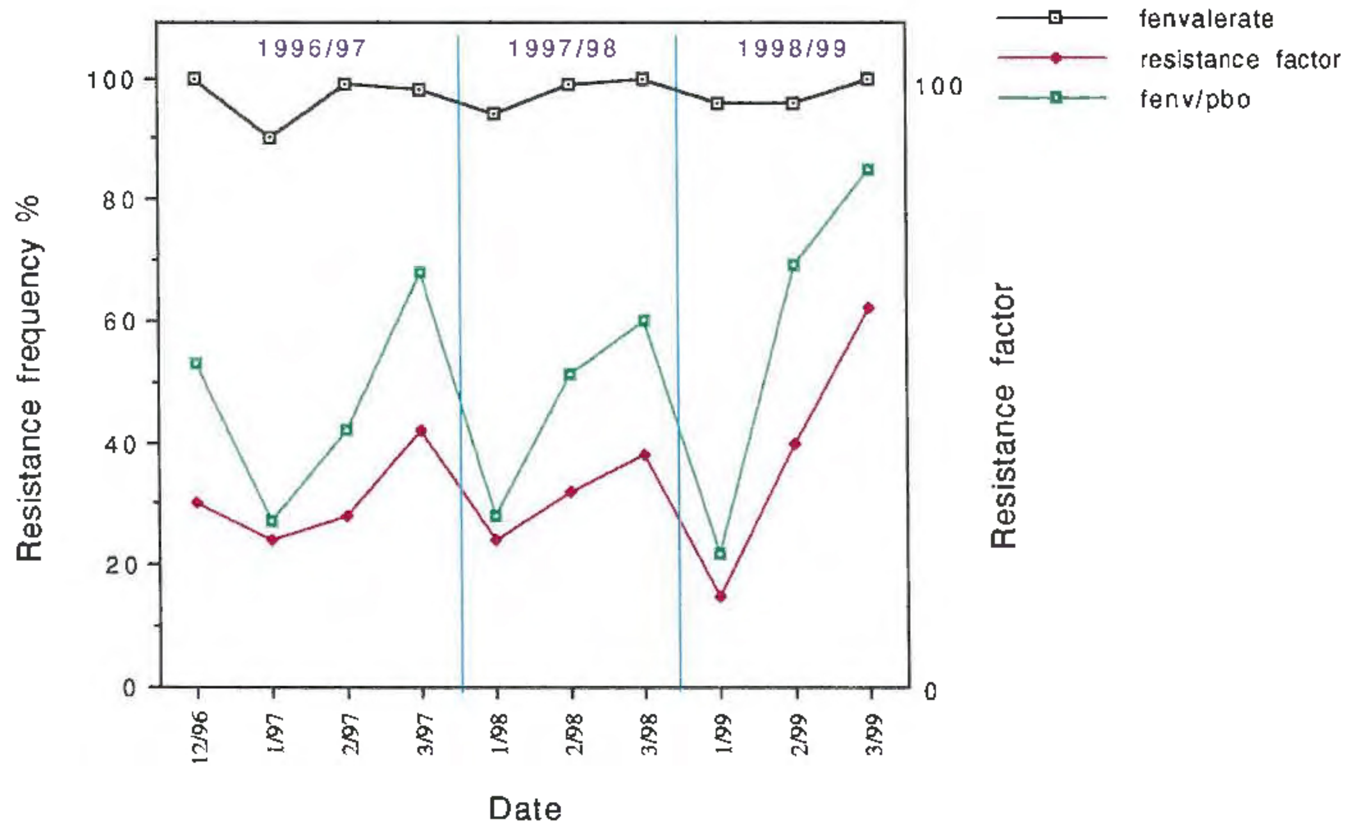


Fig. 15

Gwydir Valley - bifenthrin

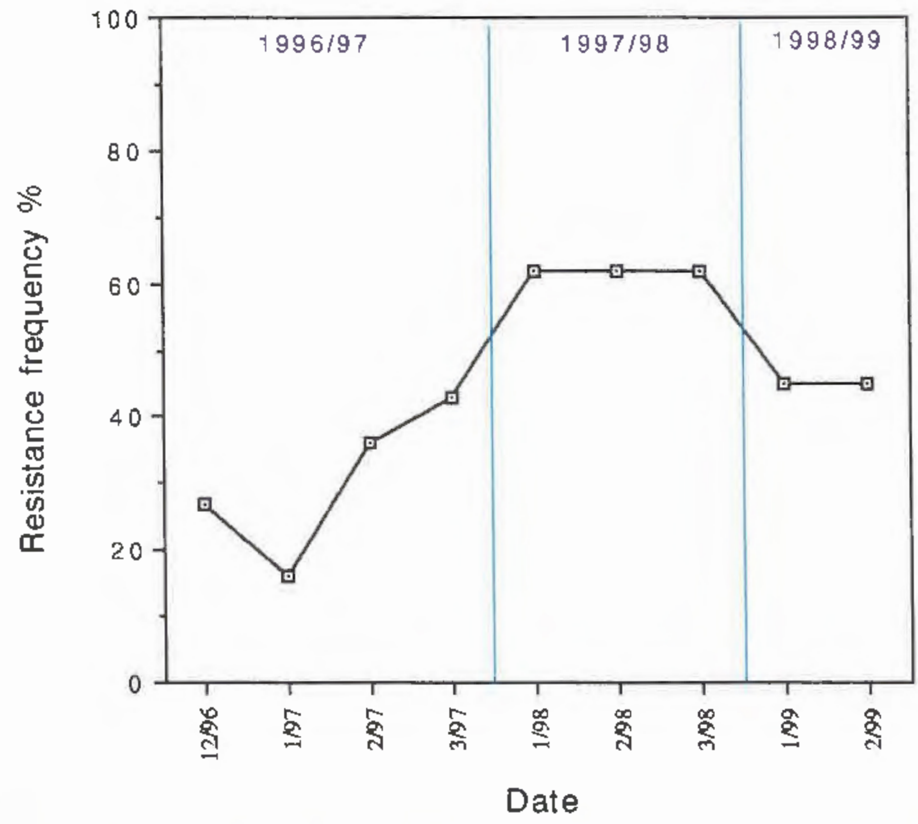


Fig. 16

Gwydir Valley - carbamates

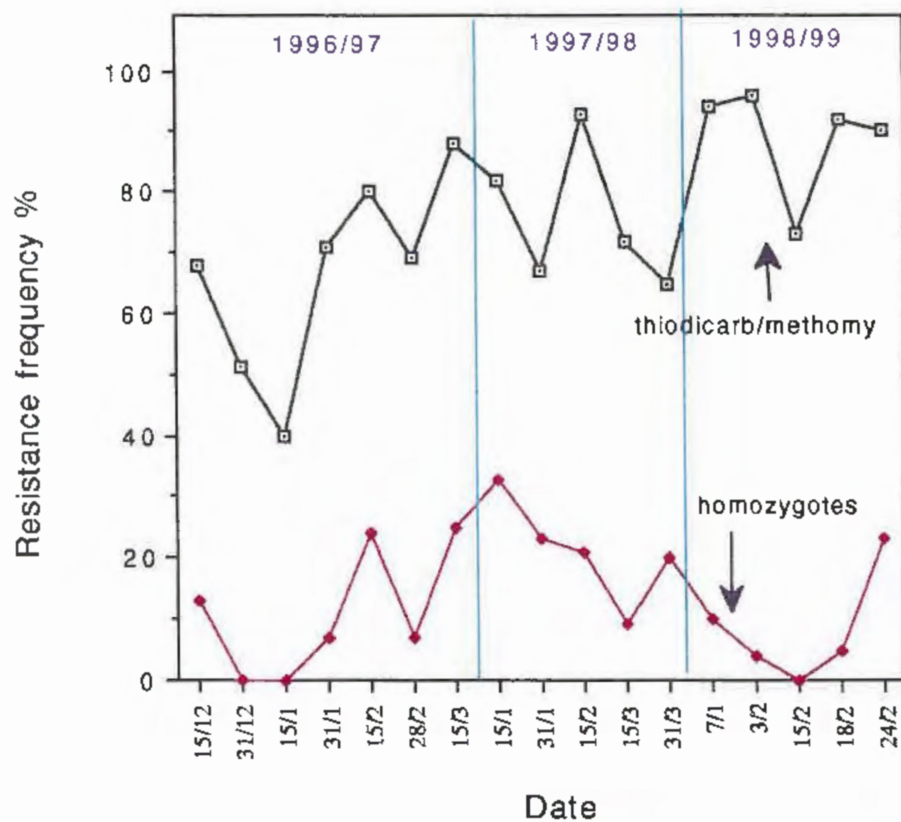


Fig. 17

Gwydir Valley - organophosphates

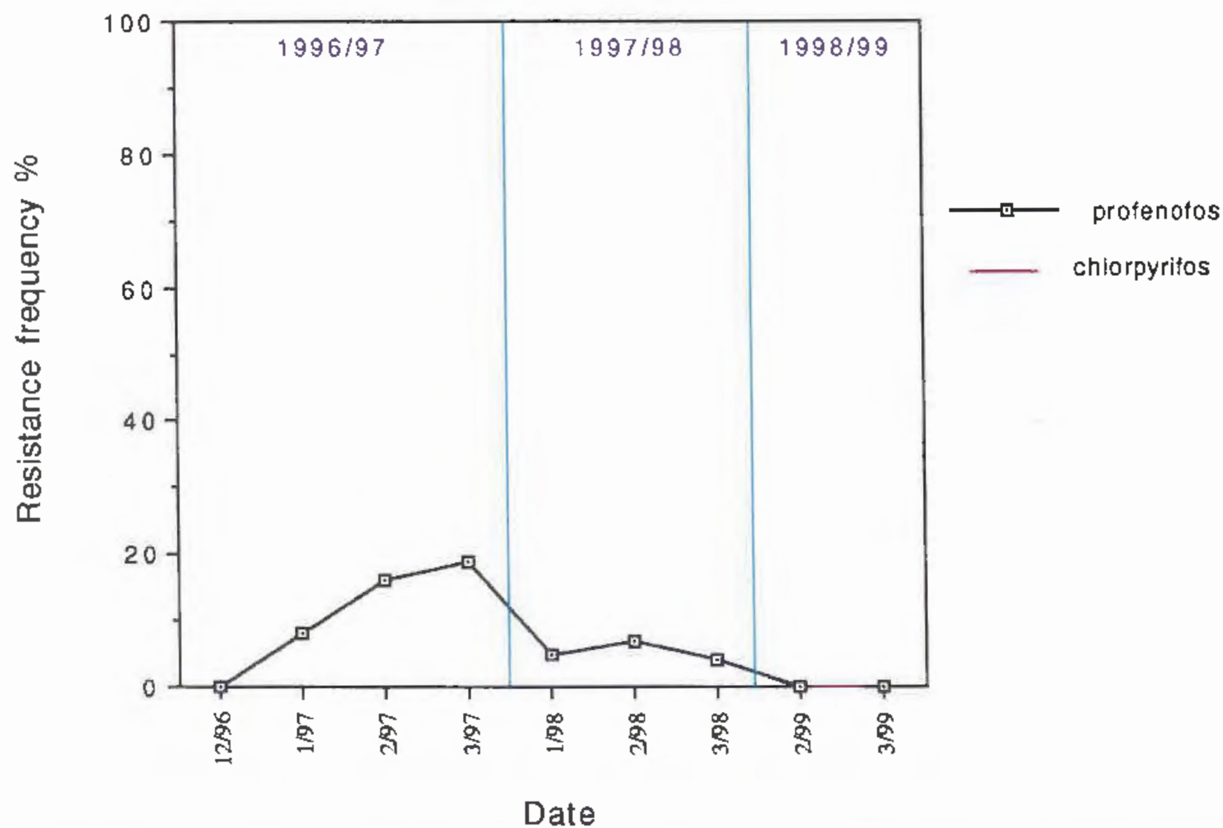


Fig. 18

Gwydir Valley - endosulfan

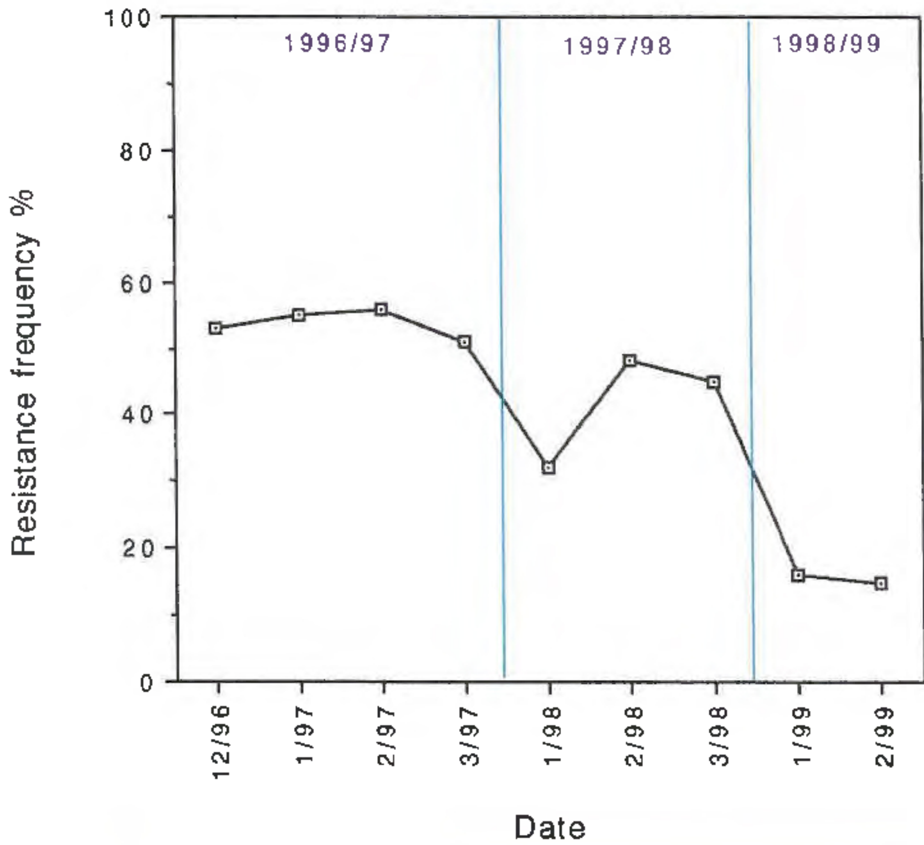


Fig. 19

Darling Downs - fenvalerate

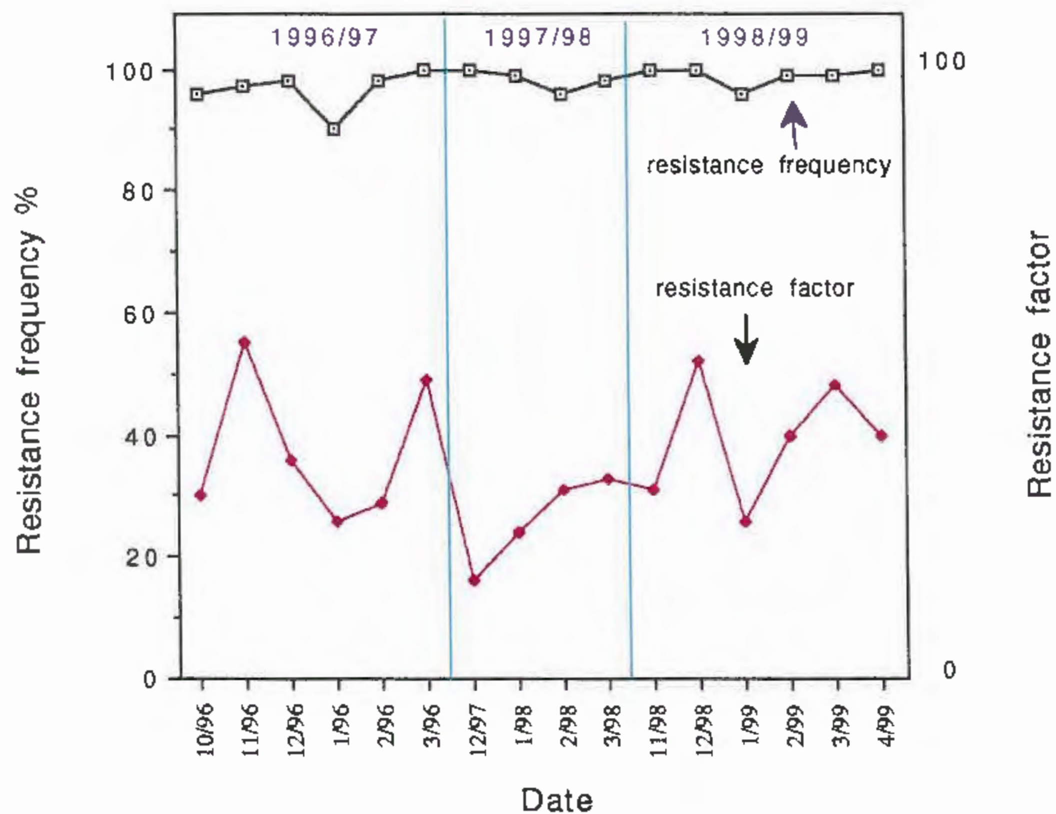


Fig. 20

Burnett - fenvalerate

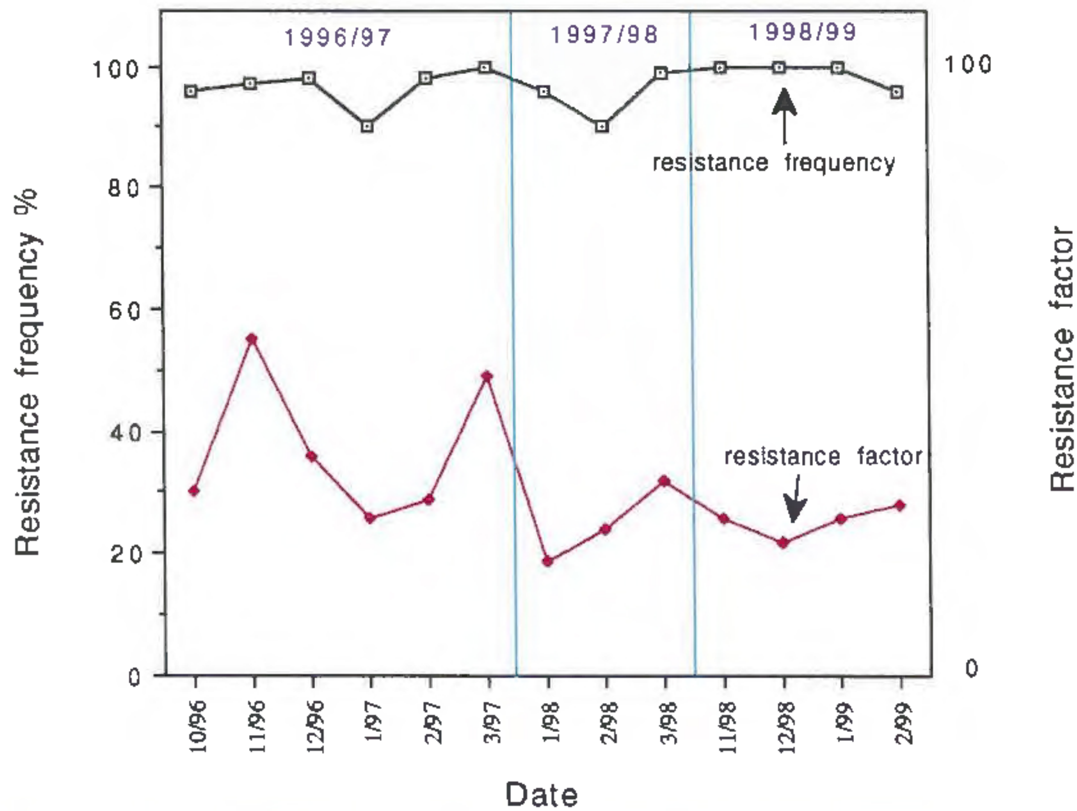


Fig. 21

Darling Downs - bifenthrin

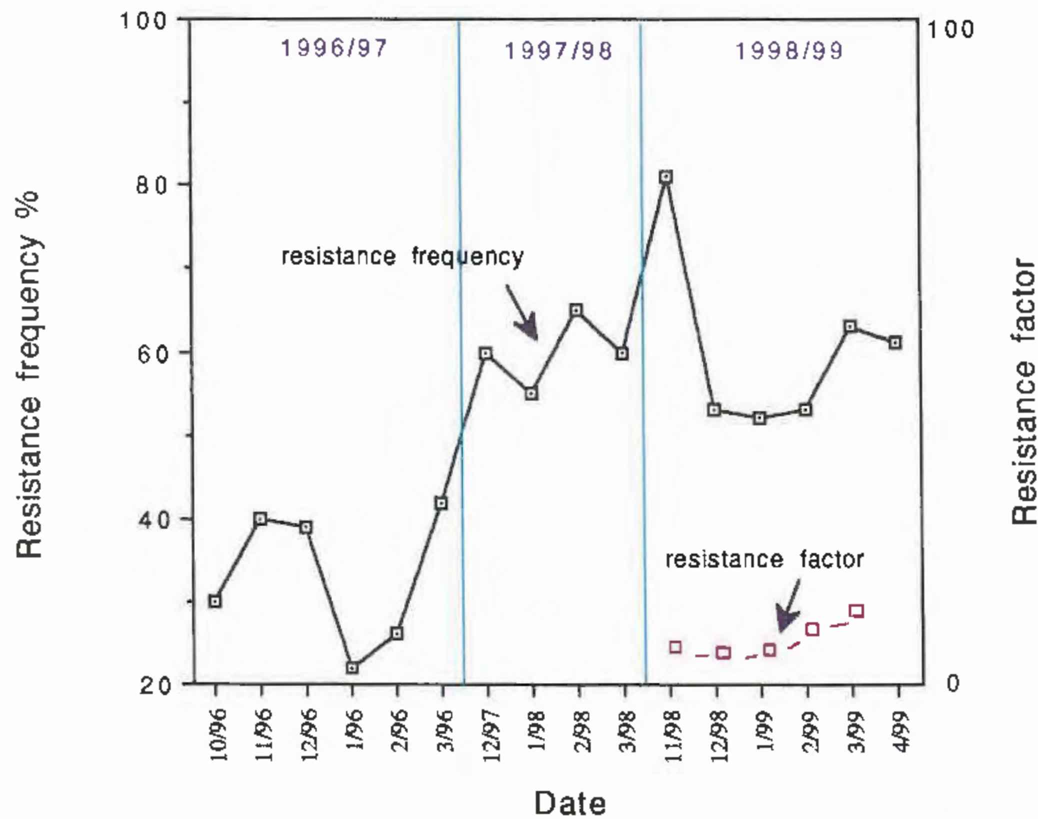


Fig. 22

Darling Downs - carbamates

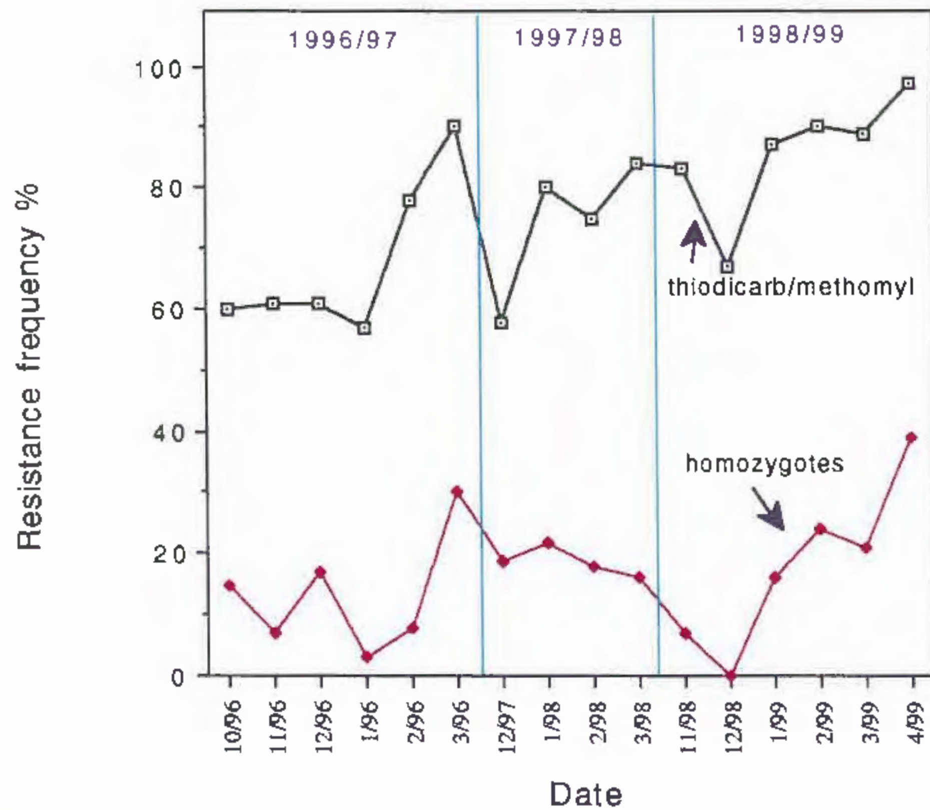


Fig. 23

Burnett - carbamates

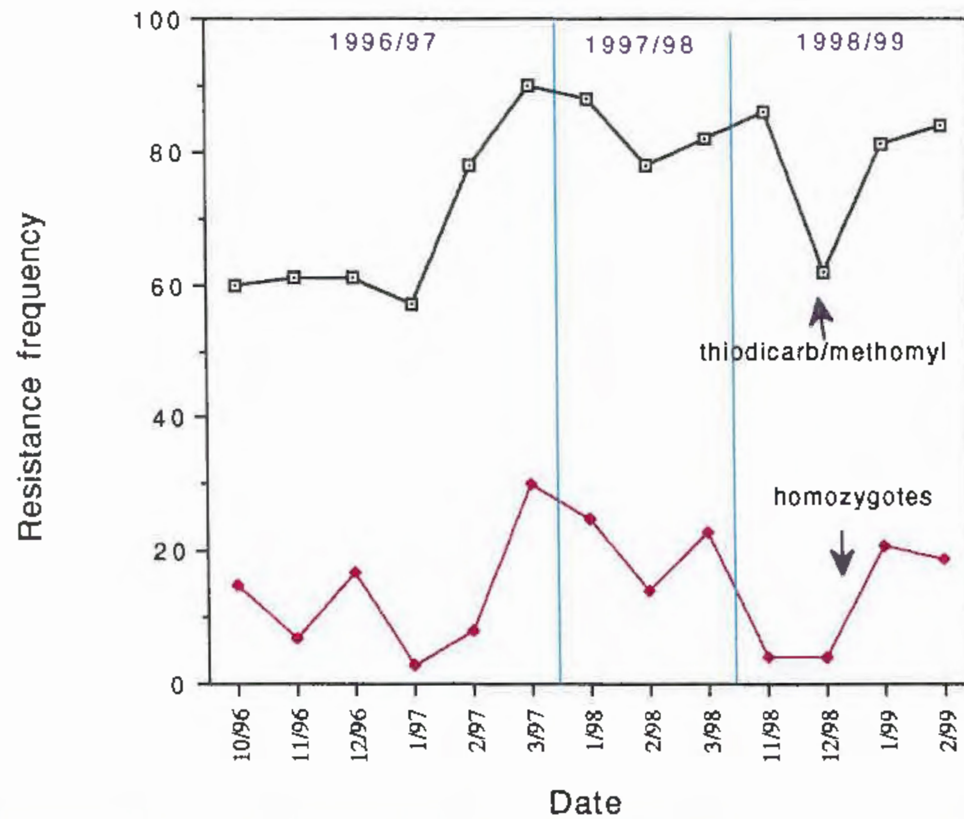


Fig. 24

Darling Downs - organophosphates

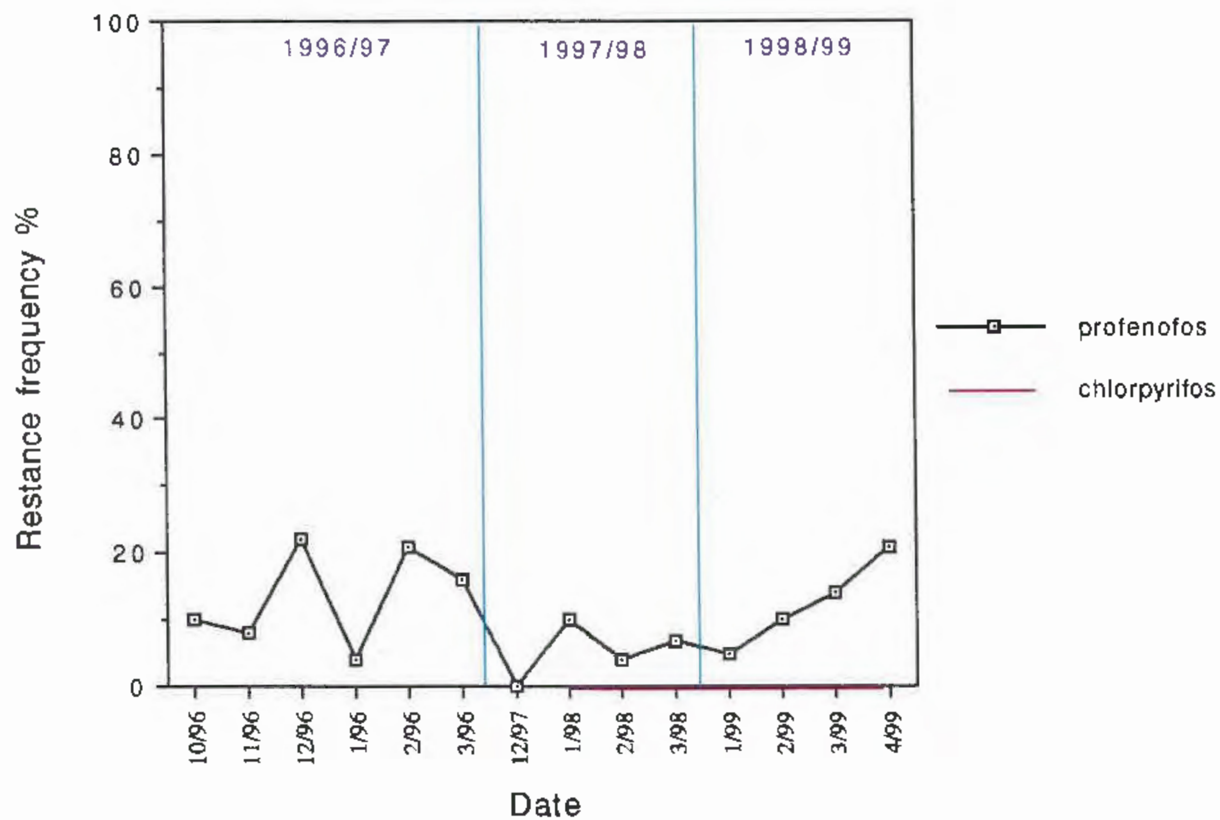


Fig. 25

Darling Downs - endosulfan

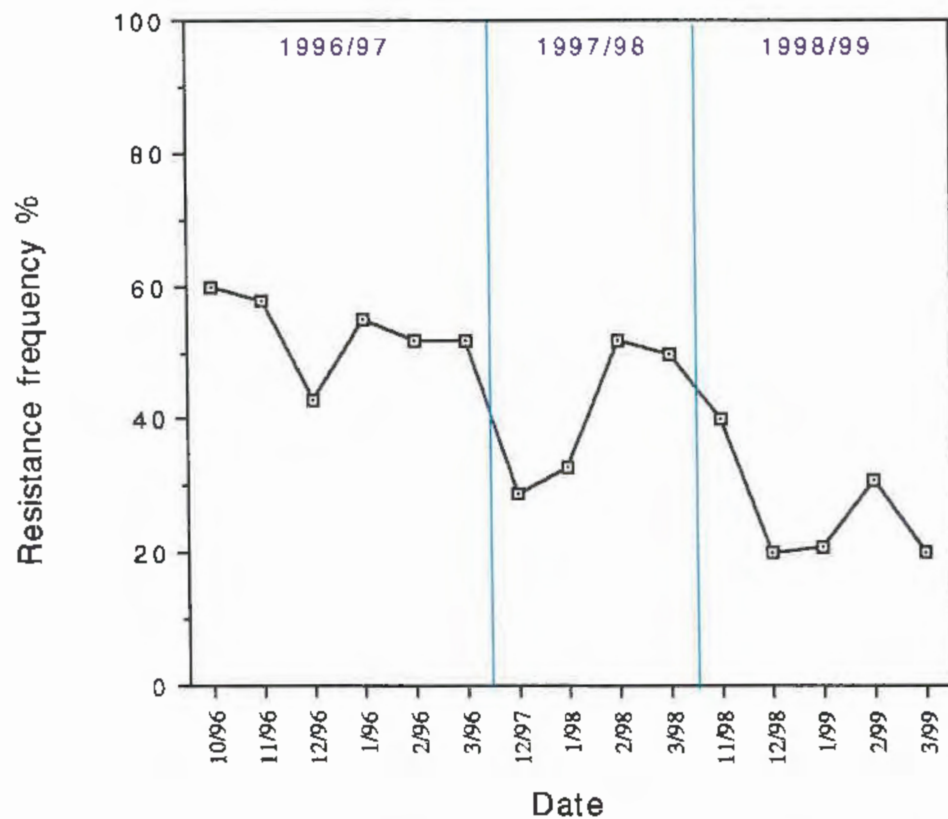


Fig. 26

Emerald - fenvalerate

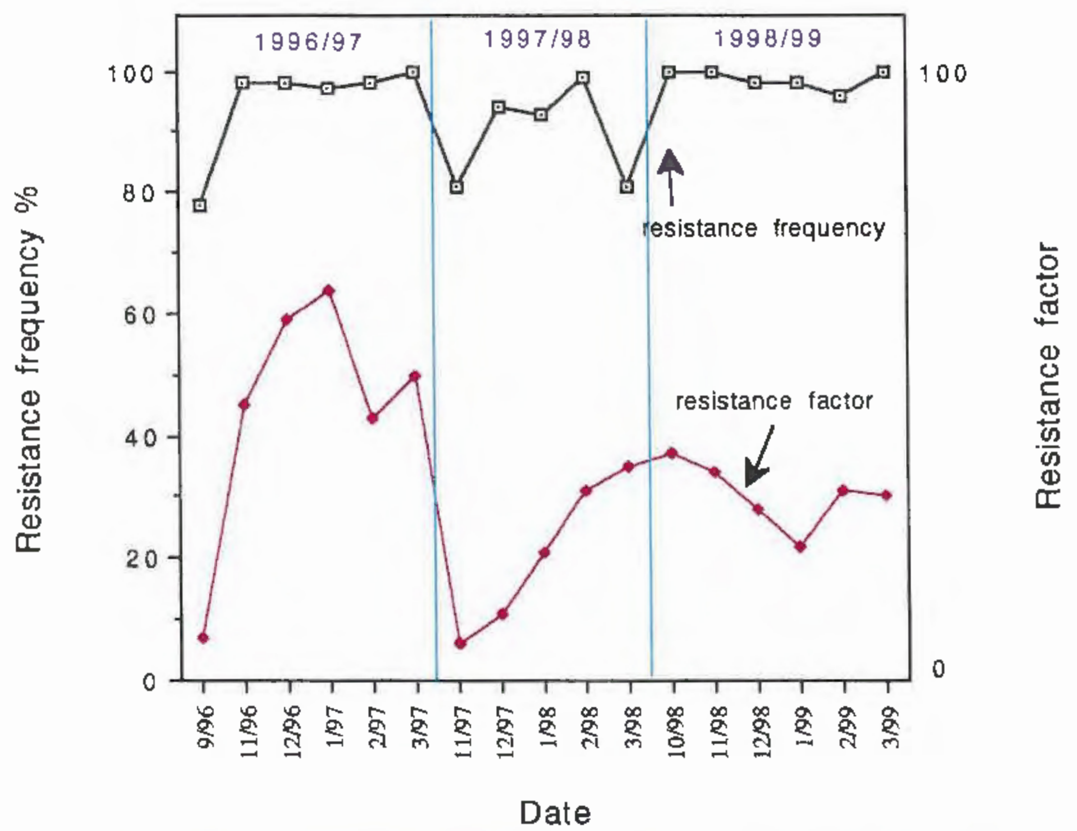


Fig. 27

Emerald - bifenthrin

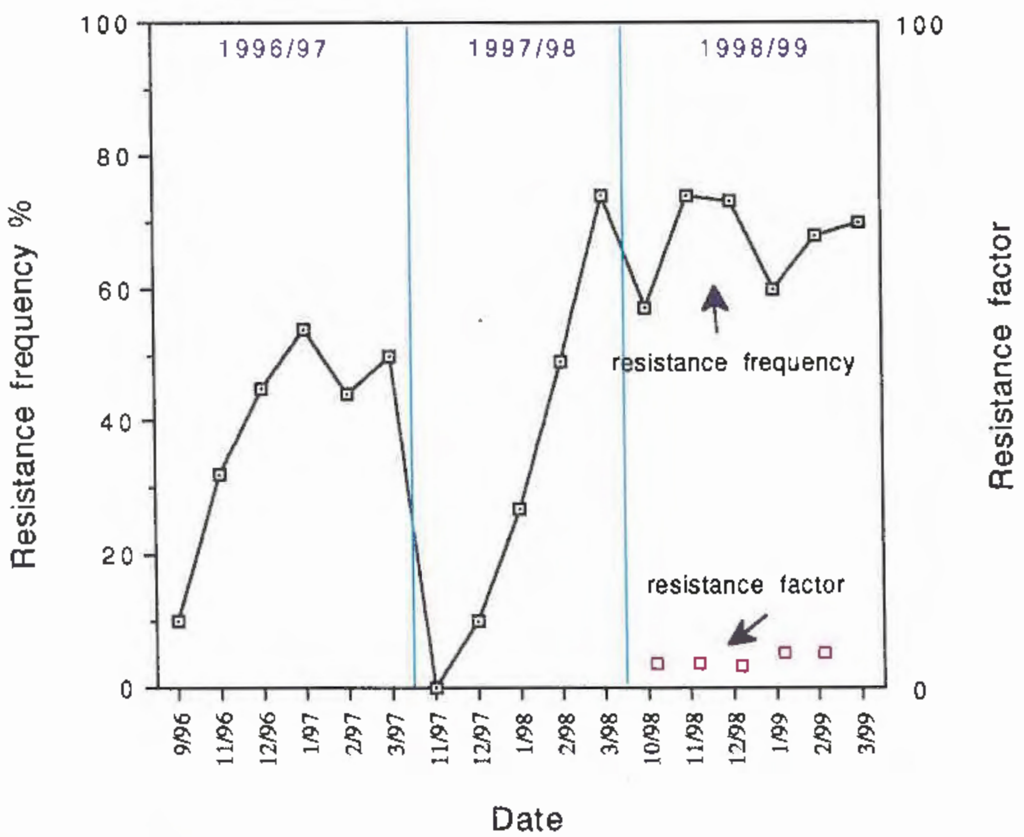


Fig. 28

Emerald - carbamates

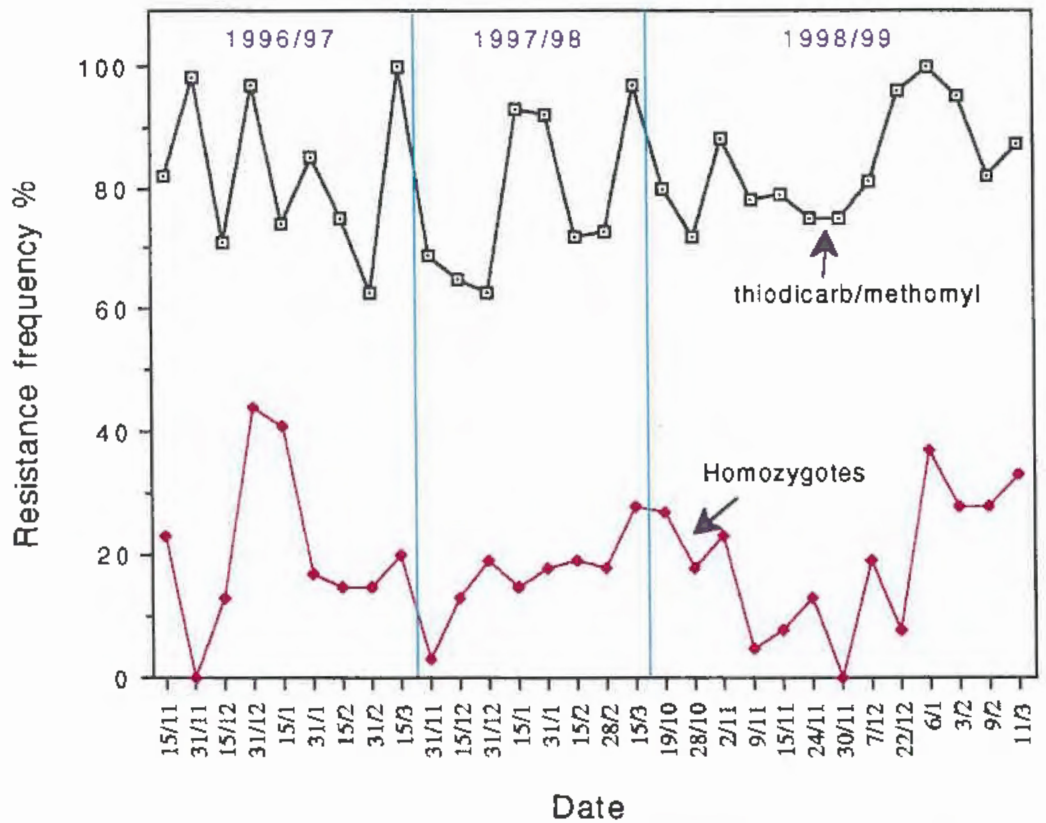


Fig. 29

Emerald - organophosphates

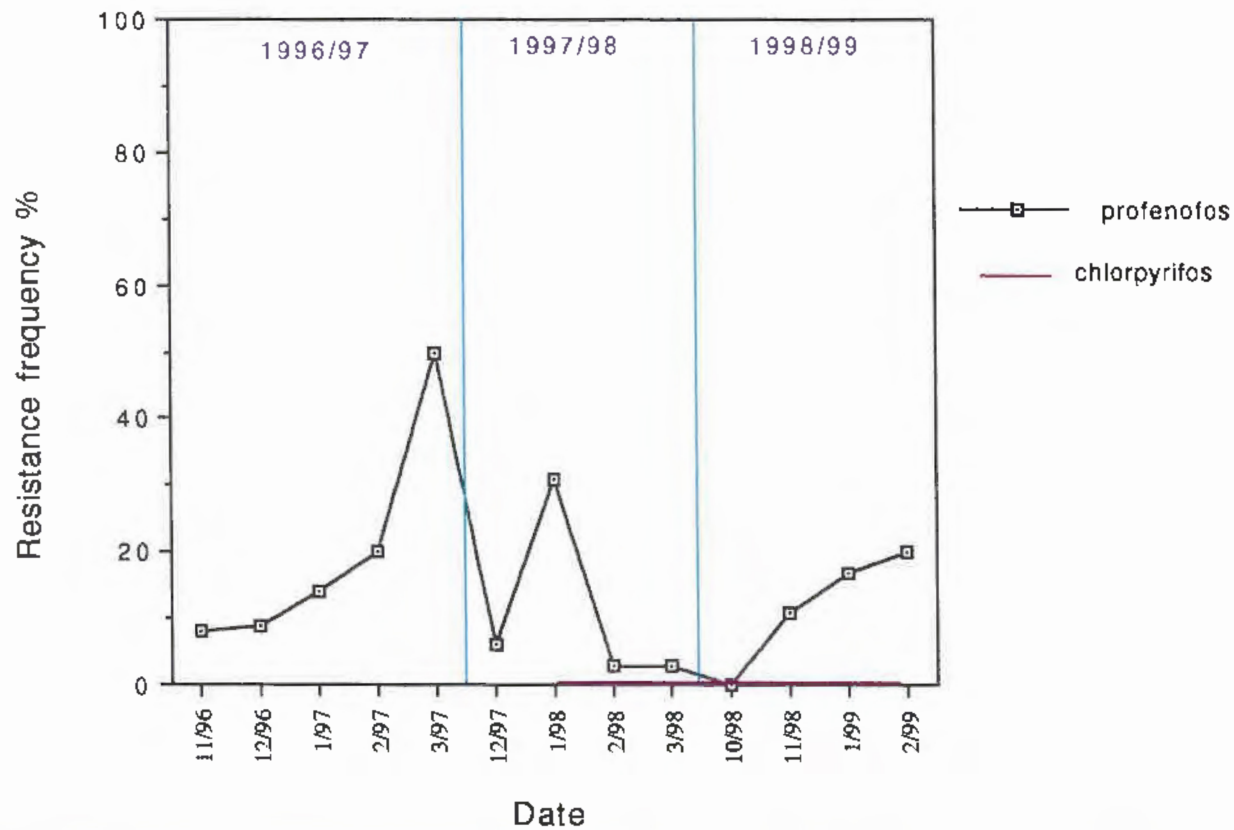


Fig. 30

Emerald - endosulfan

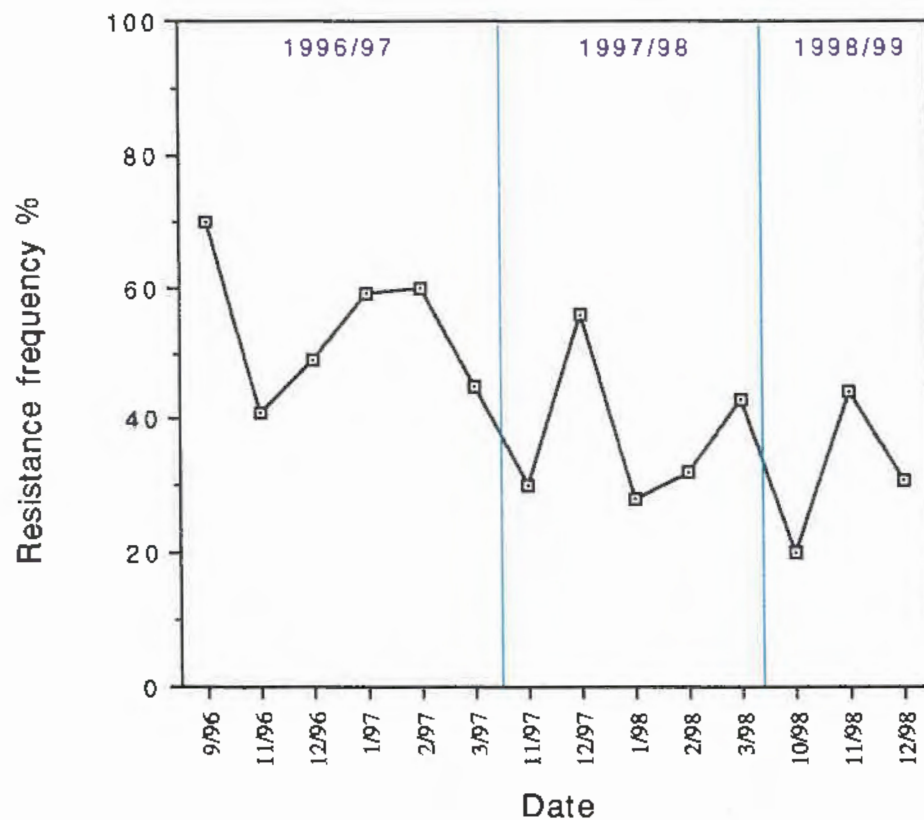


Fig. 31

Emerald - other compounds 98/99

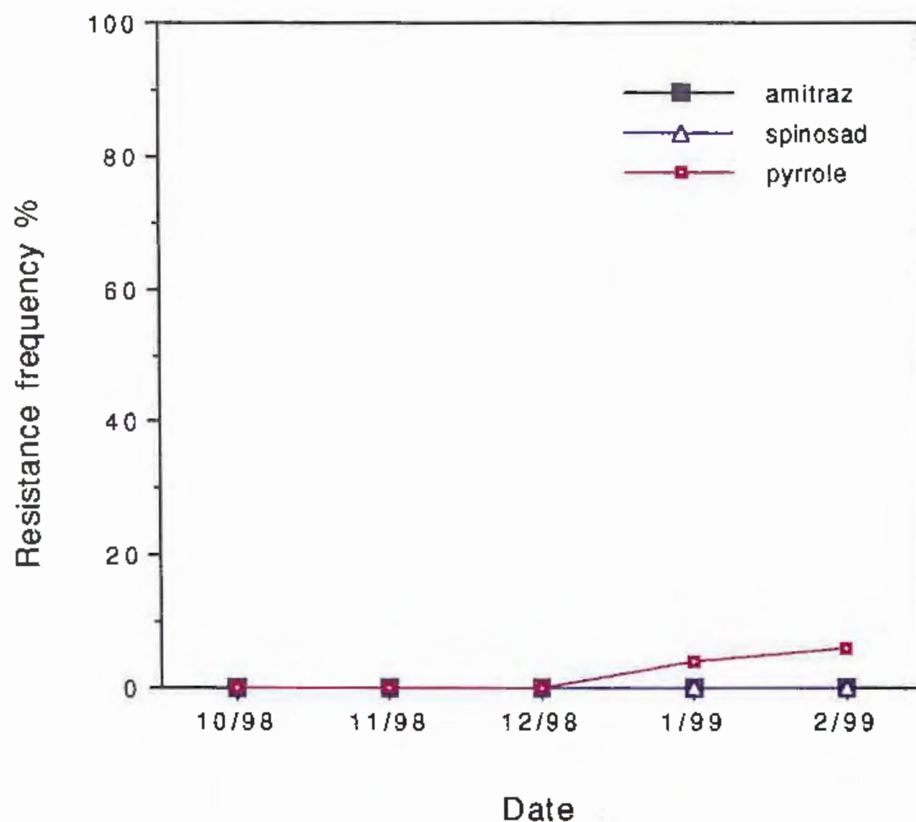


Fig. 32

St George - fenvalerate

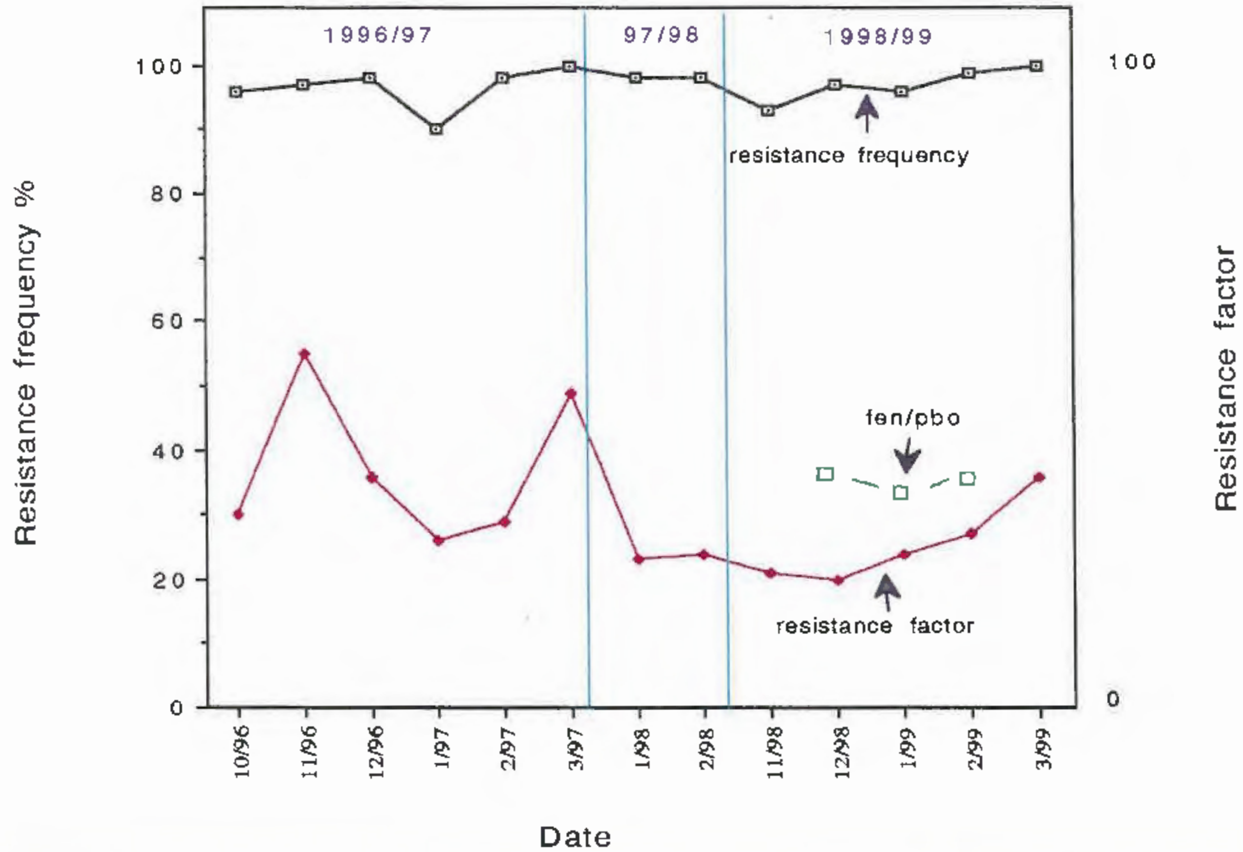


Fig. 33

St George - bifenthrin

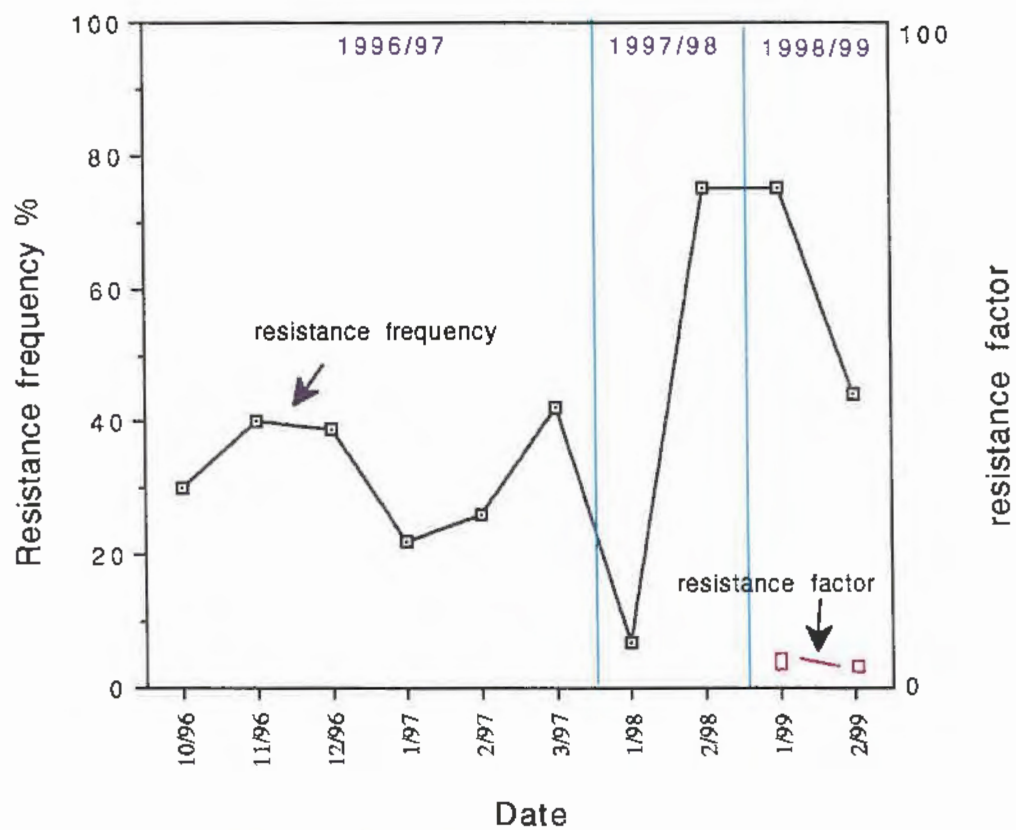


Fig. 34

St George - carbamates

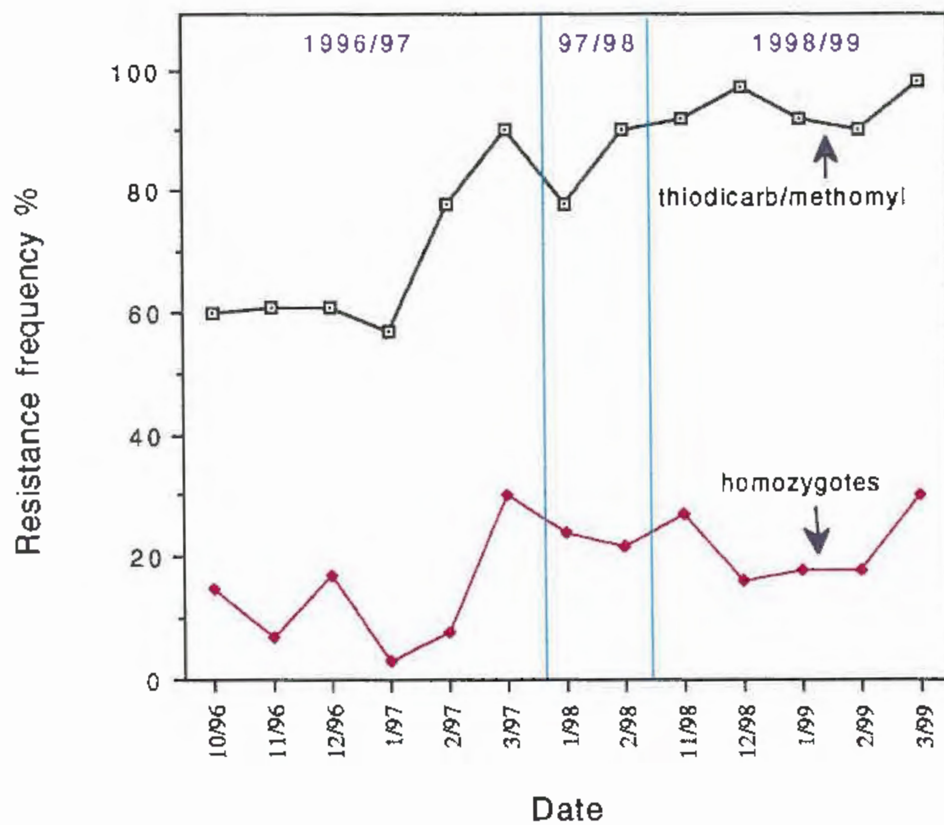


Fig. 35

St George - organophosphates

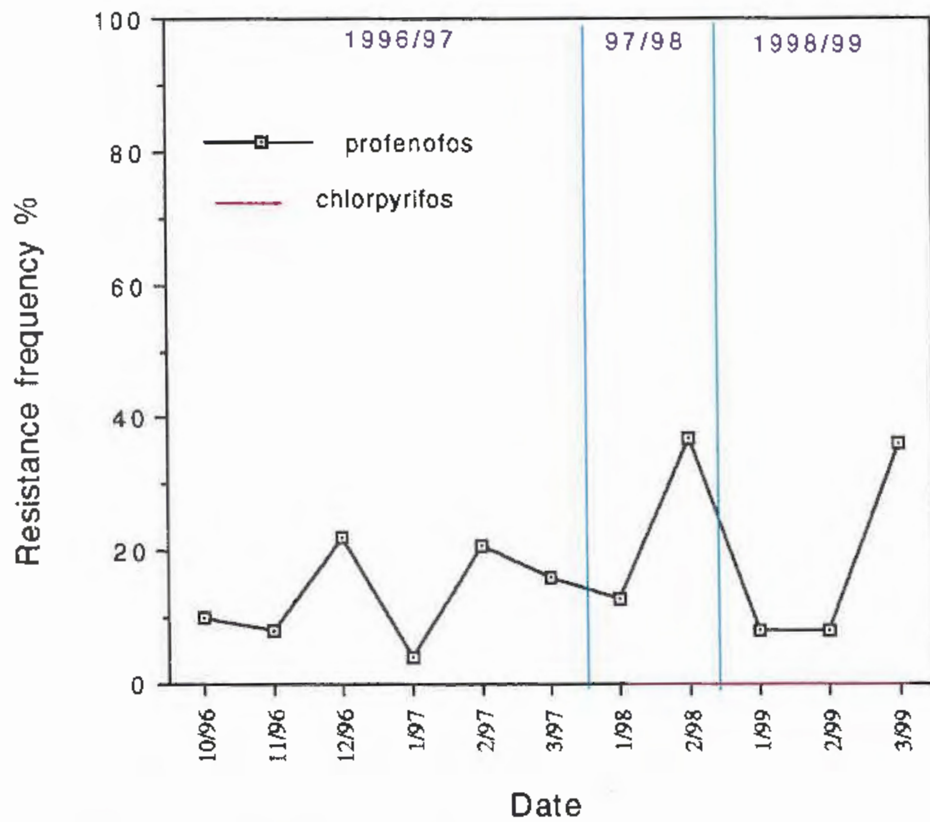


Fig. 36

St George - endosulfan

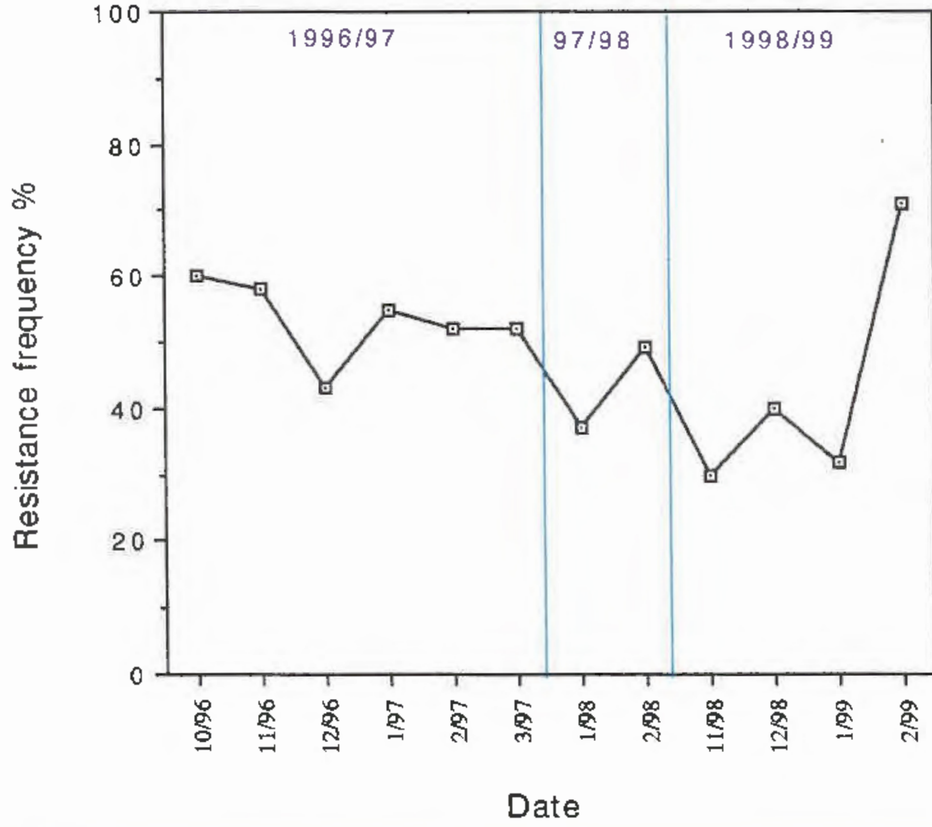


Fig. 37

St George - other compounds

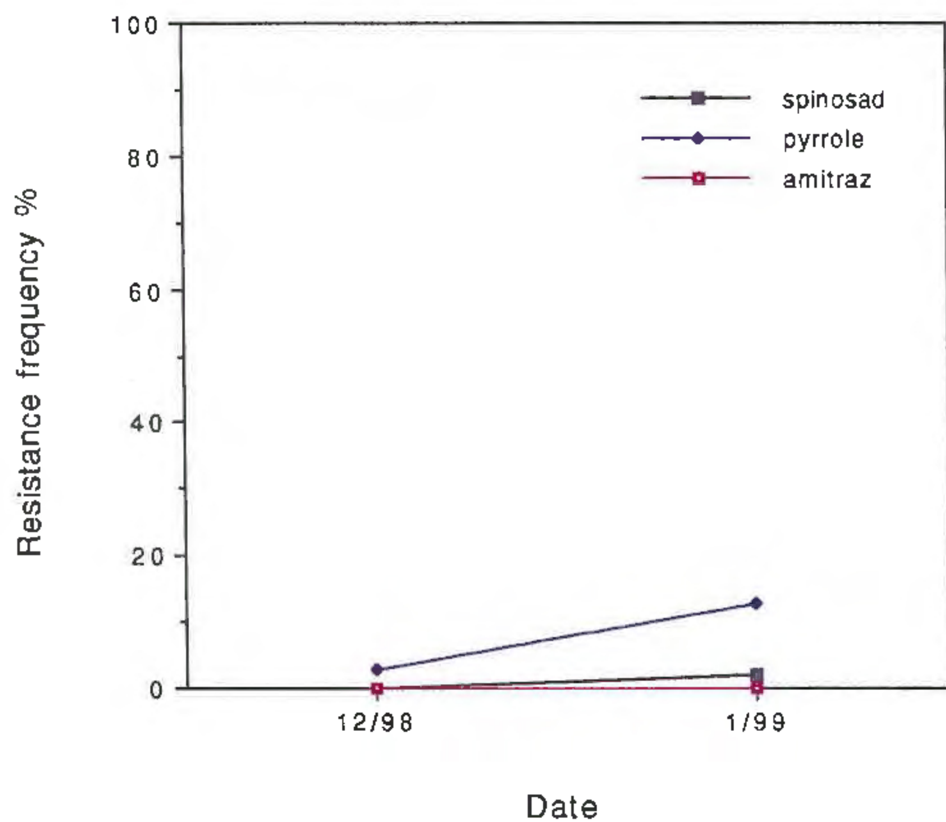


Fig. 38

Theodore / Bileola - fenvalerate

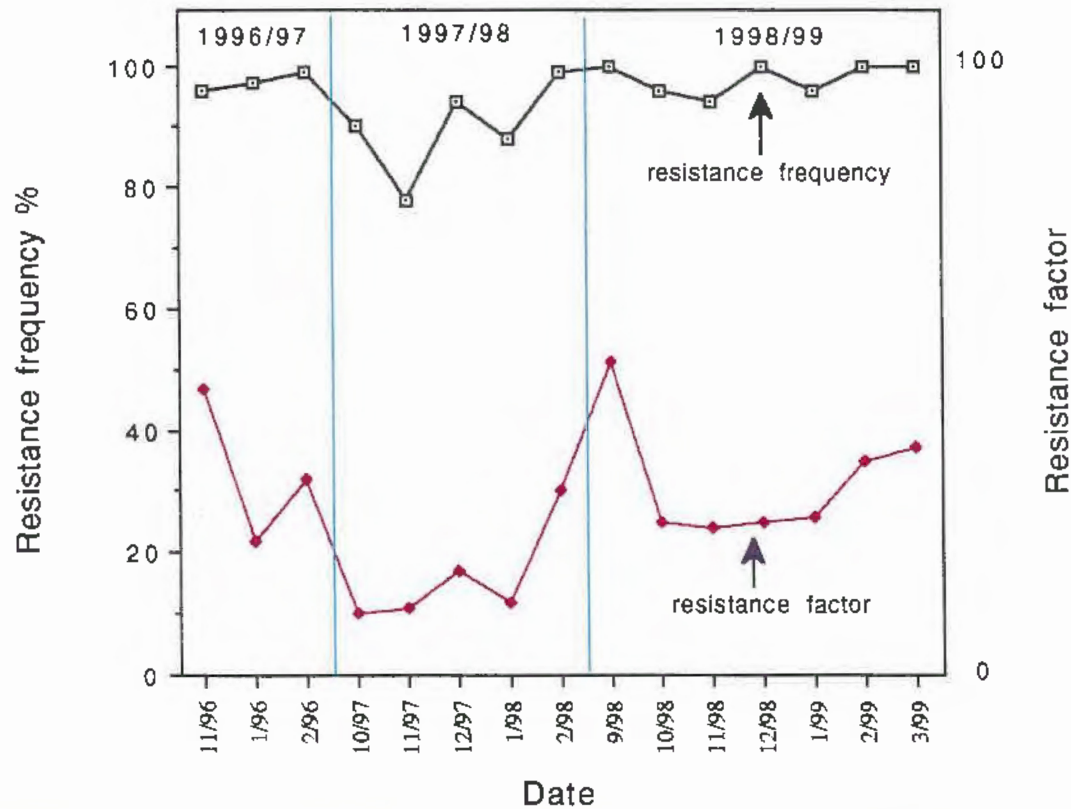


Fig. 39

Theodore / Biloela - bifenthrin

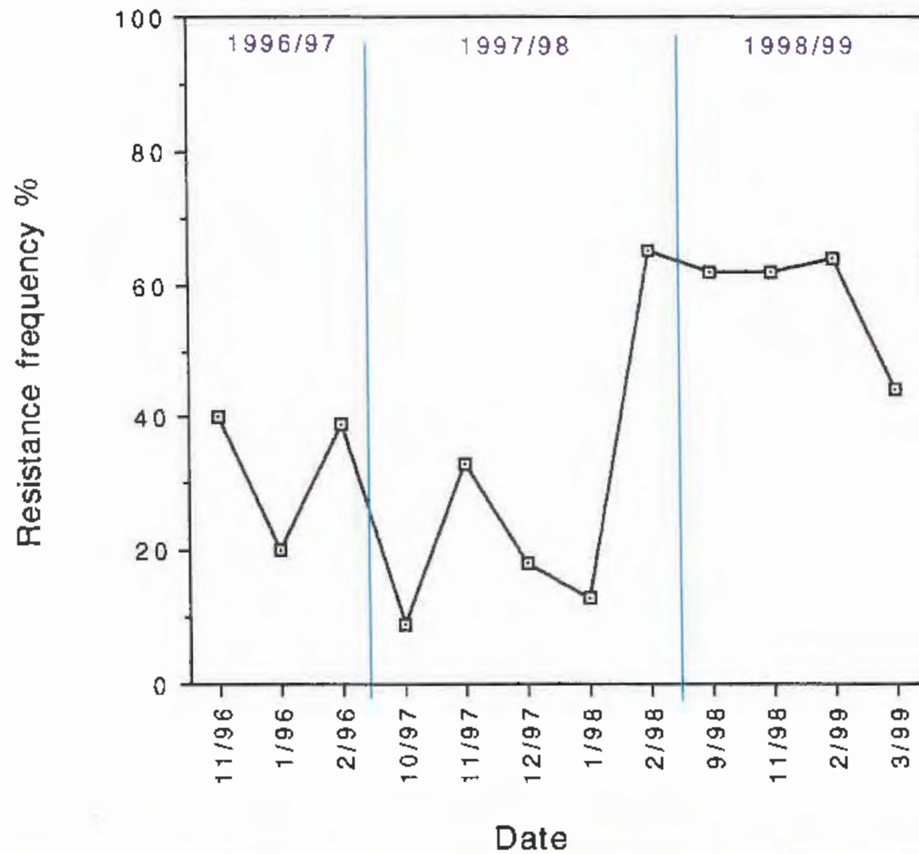


Fig. 40

Theodore / Biloela - carbamates

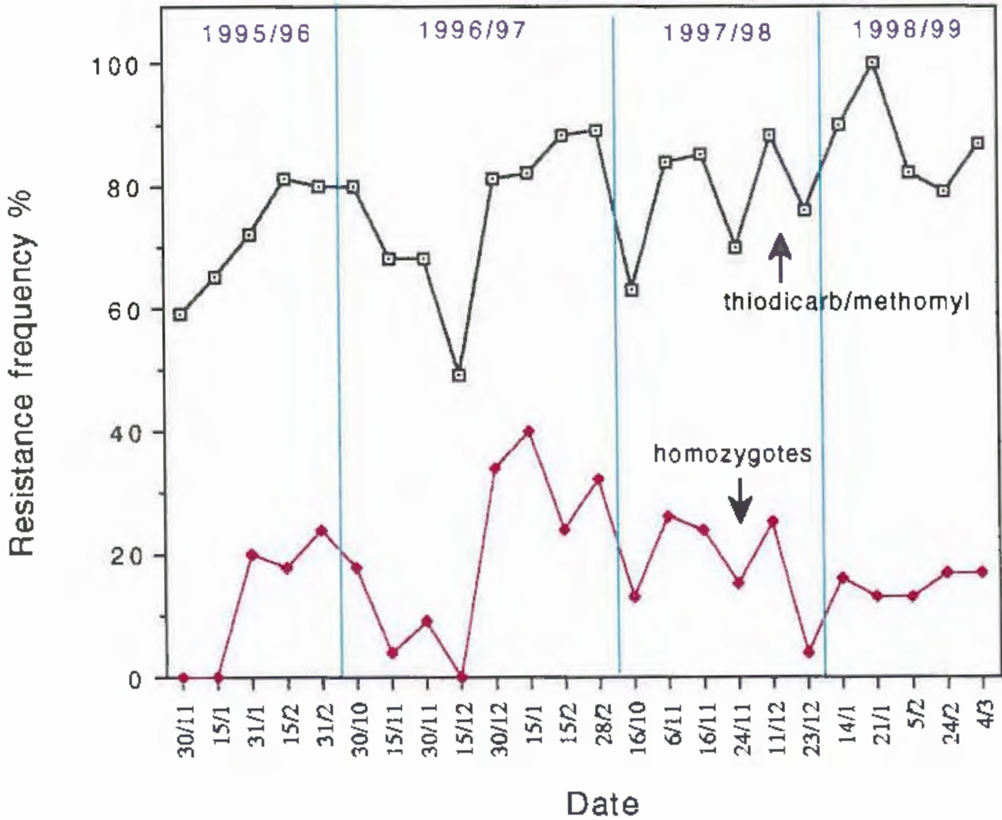


Fig. 41

Theodore / Biloela organophosphates

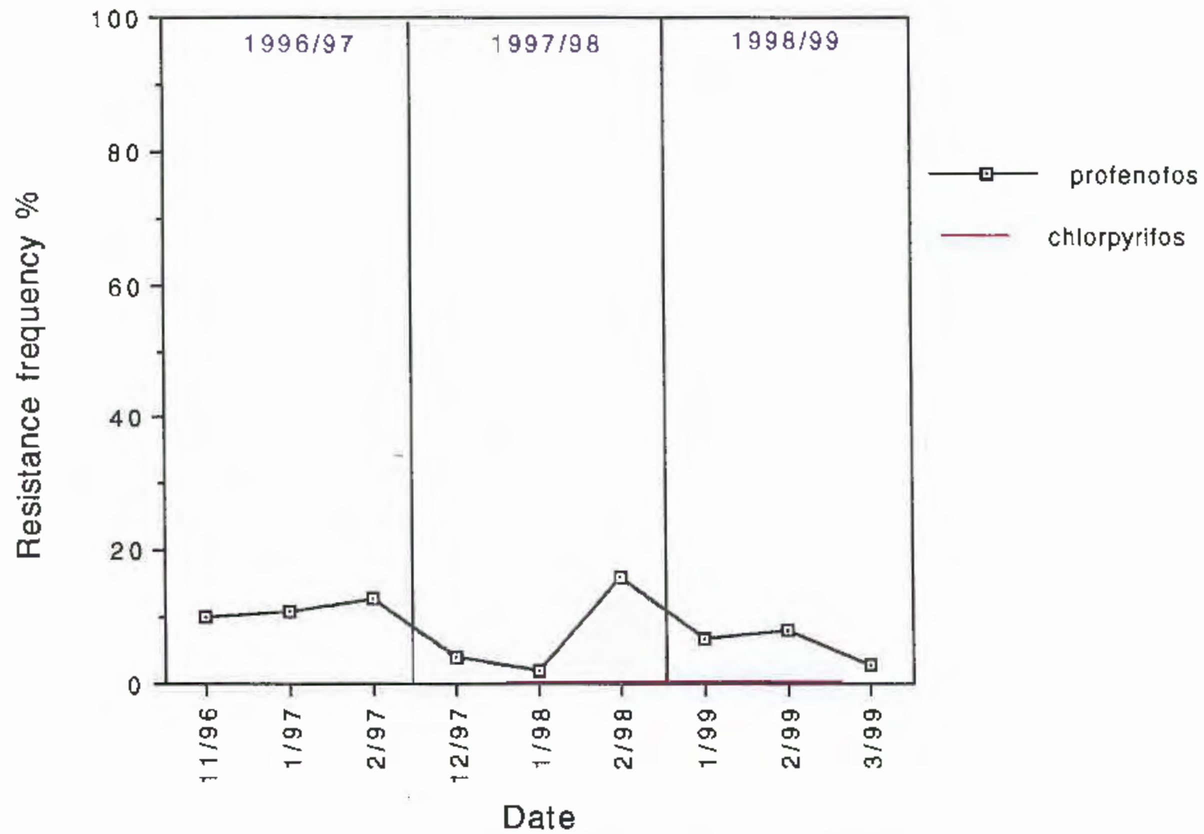


Fig. 42

Macintyre - fenvalerate

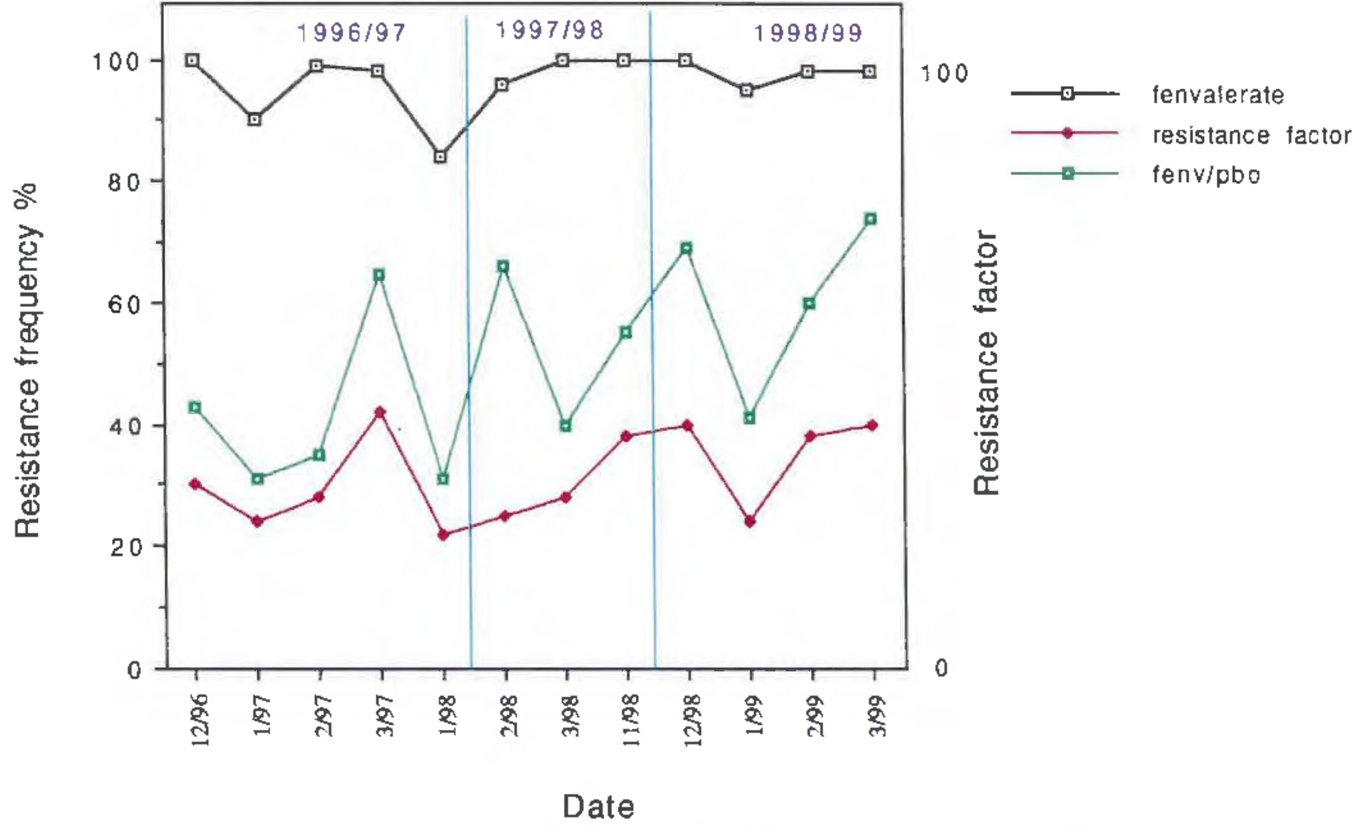


Fig. 43

Macintyre - bifenthrin

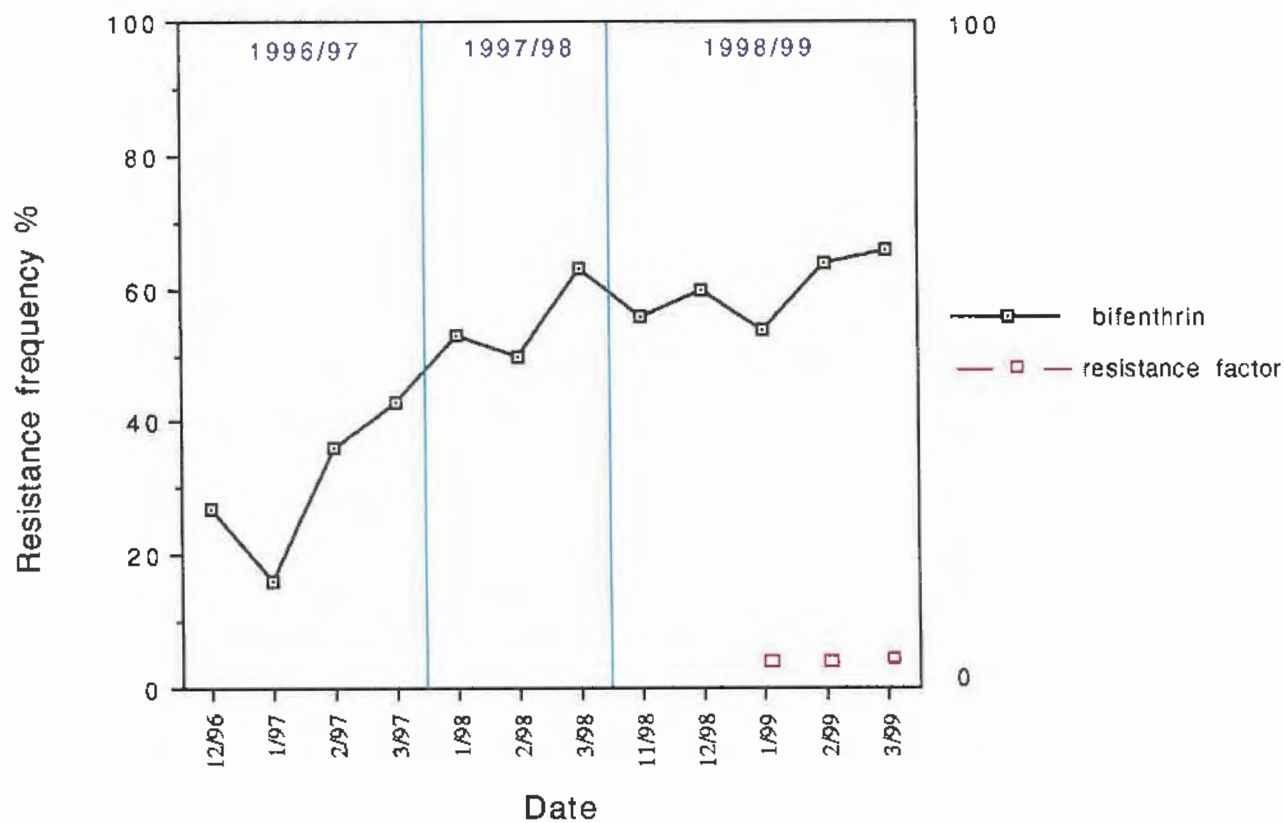


Fig. 44

Macintyre - carbamates

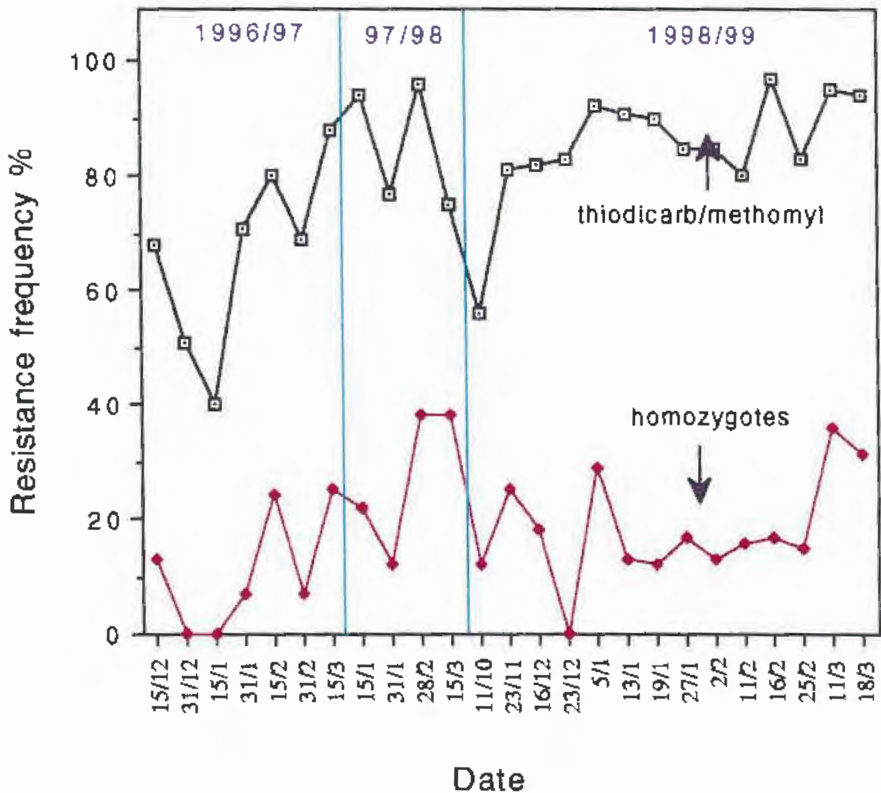


Fig. 45

Macintyre - organophosphates

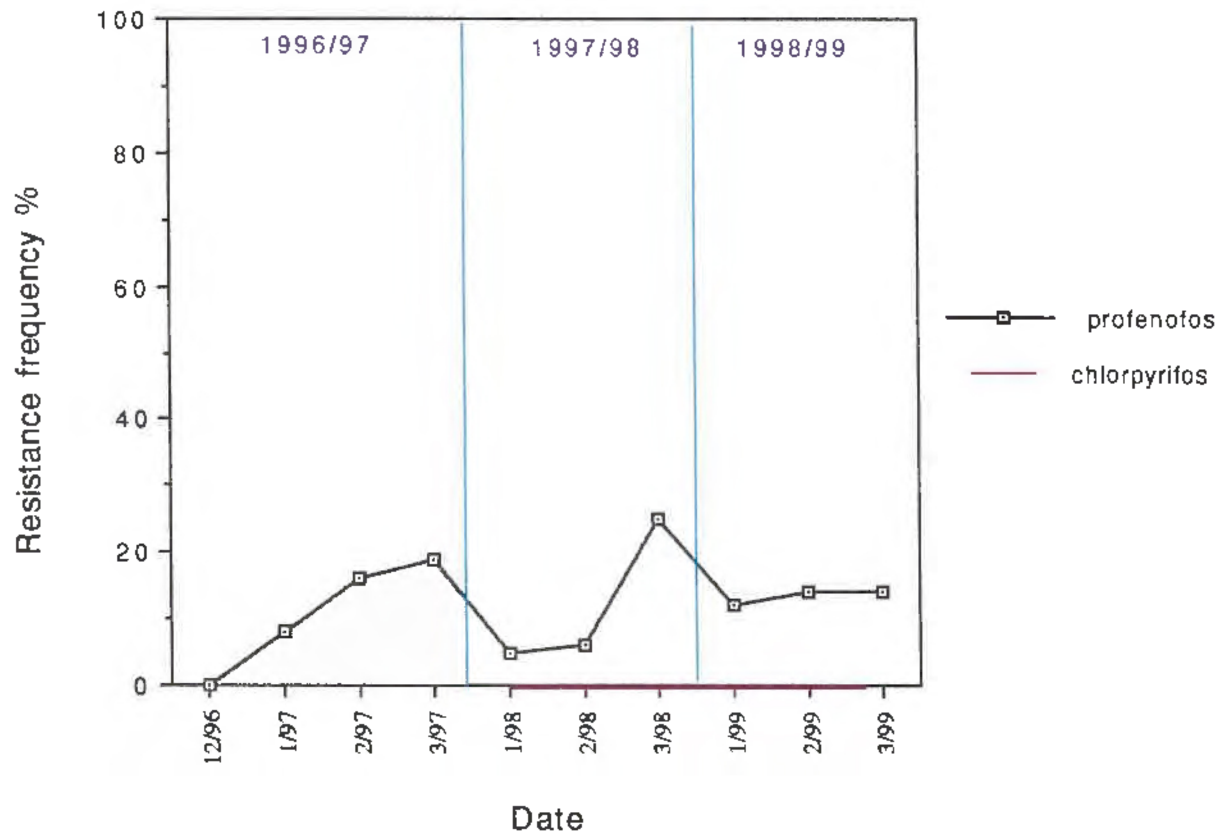


Fig. 46

Macintyre - endosulfan

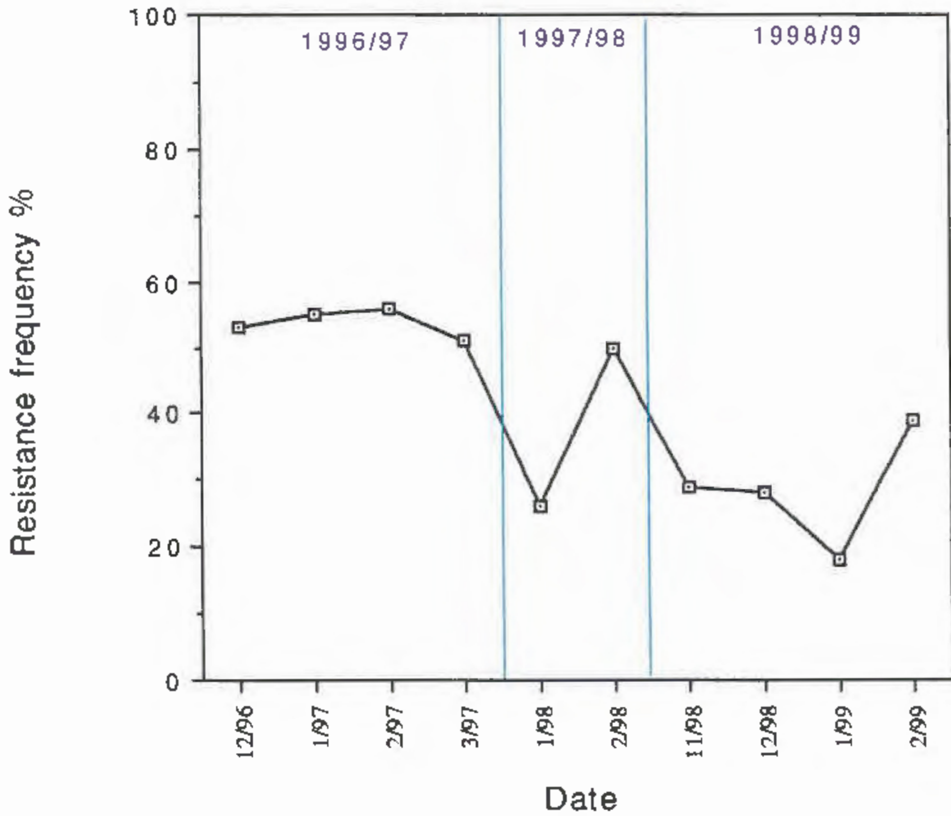


Fig. 47

Macintyre - other compounds 98/99

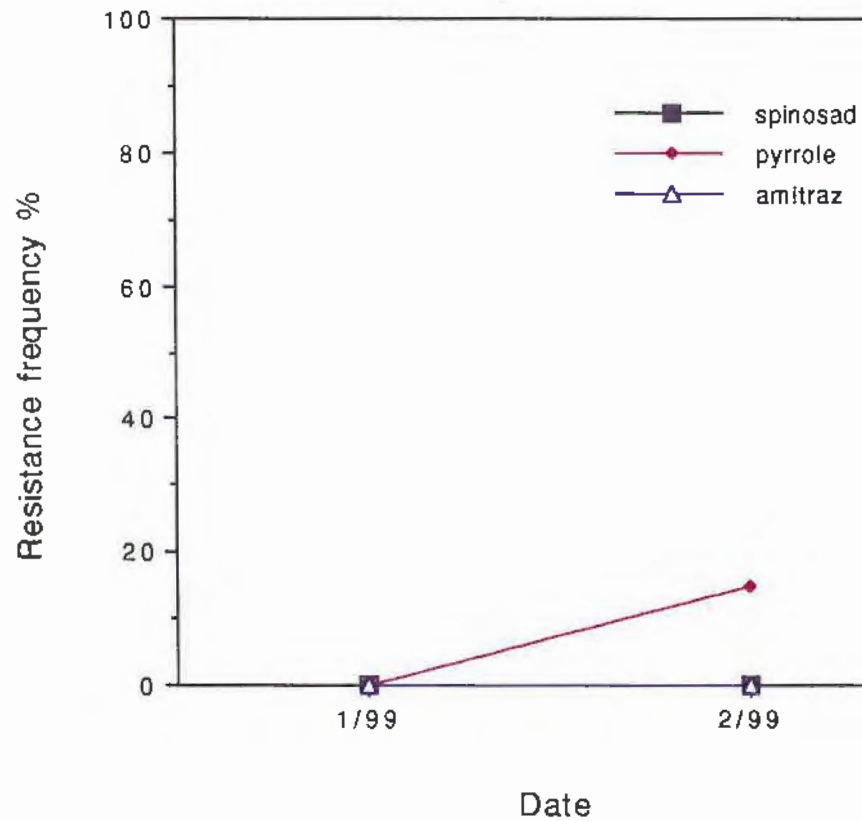


Fig. 48

Bourke - fenvalerate 1998/99

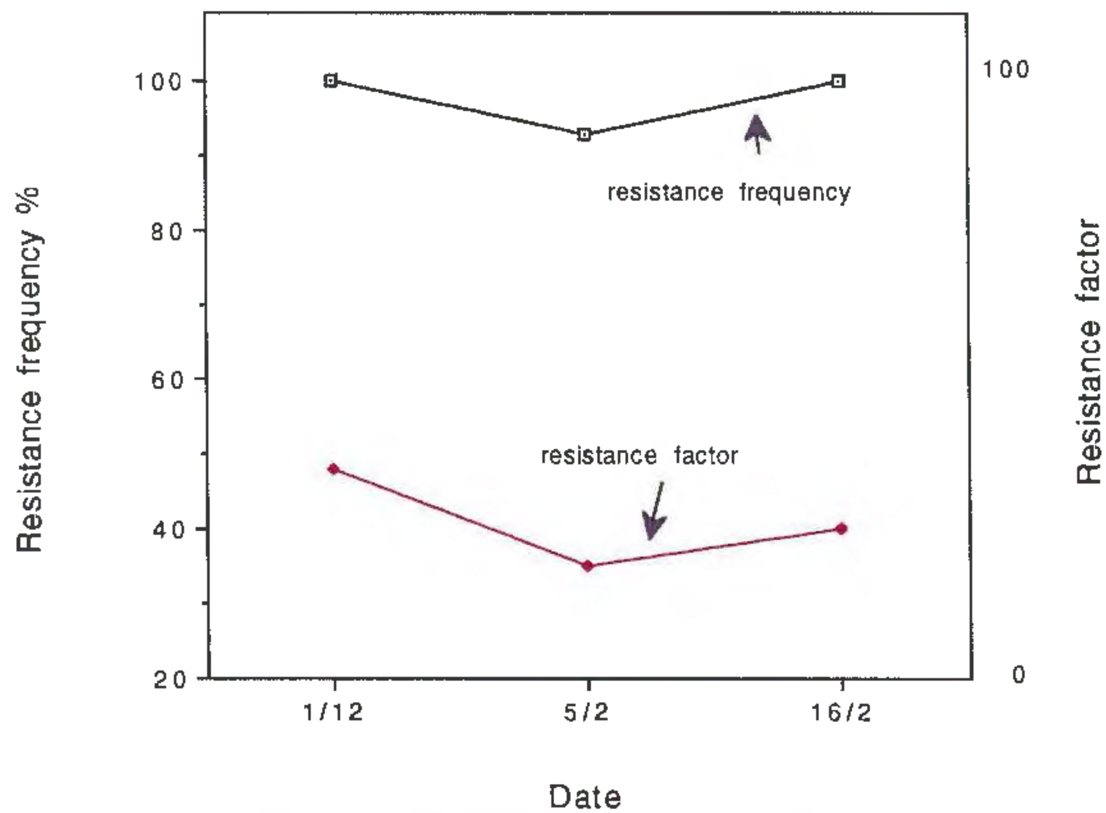


Fig. 49

Bourke - Carbamates 1998/99

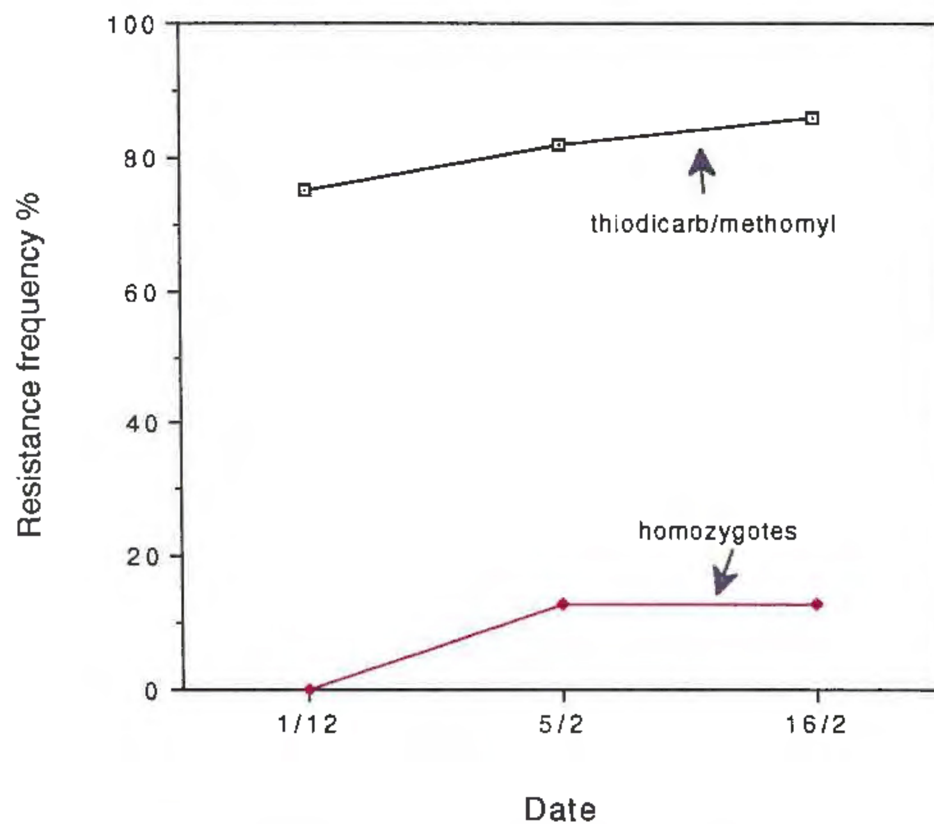


Fig. 50

Acetylcholine esterase inhibition by methomyl in resistant *H. armigera*

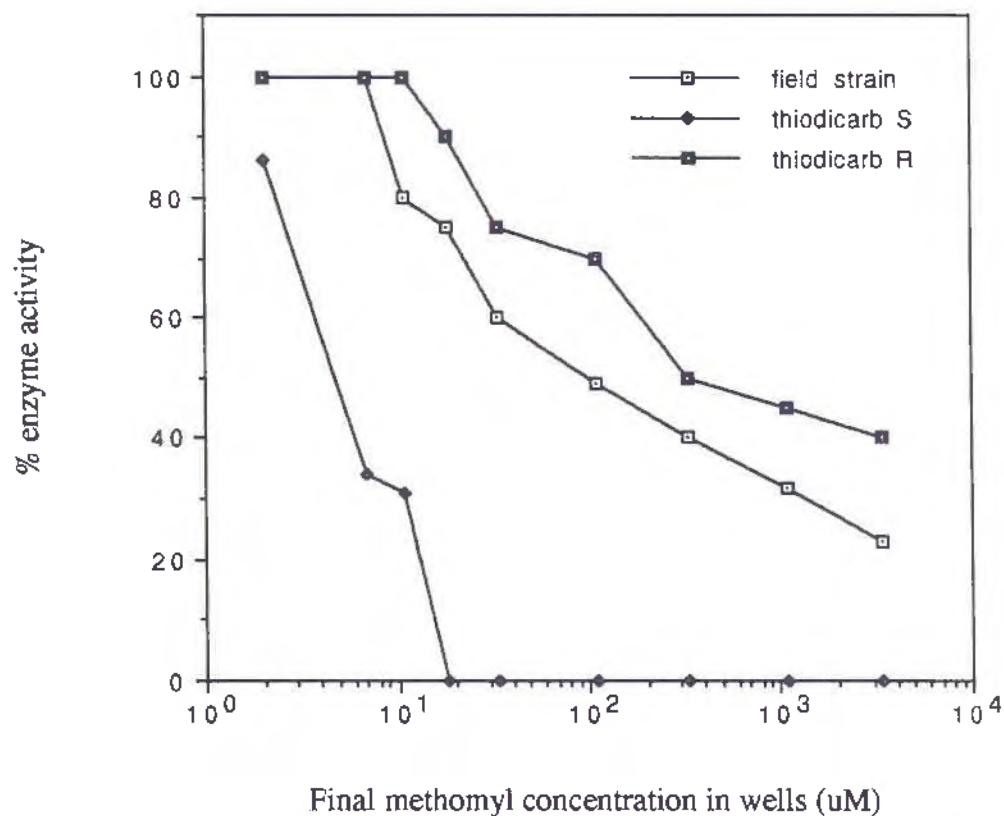


Fig. 51

Acetylcholine esterase inhibition by thiodicarb in resistant *H. armigera*

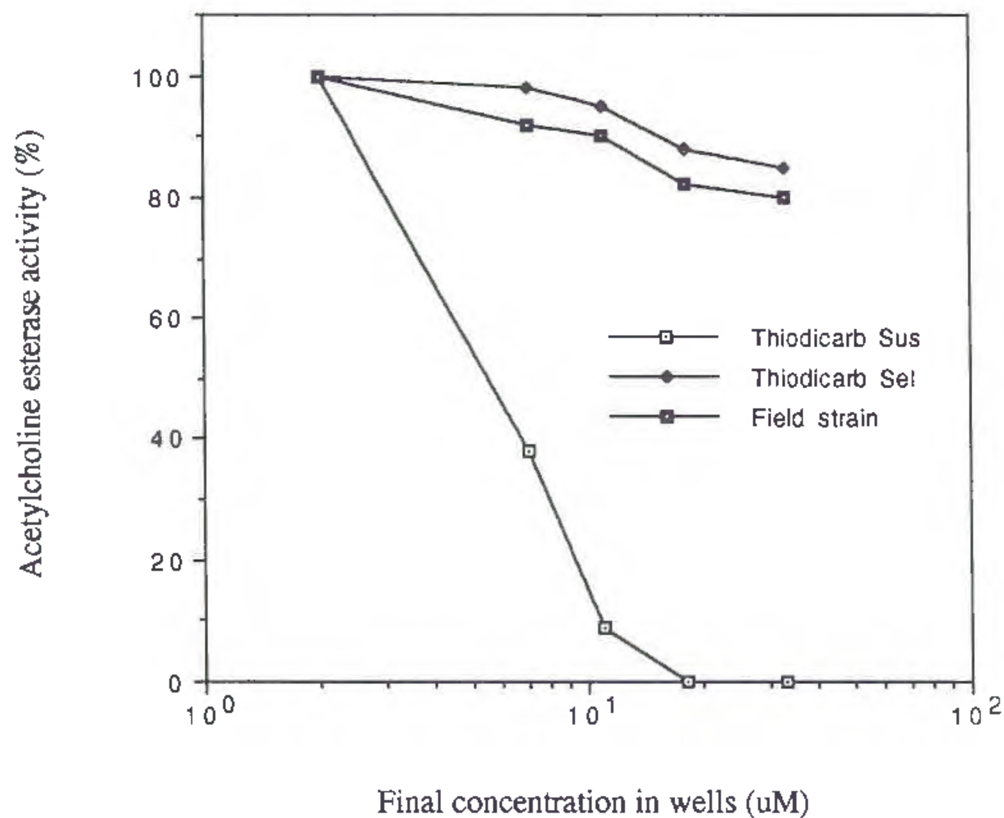
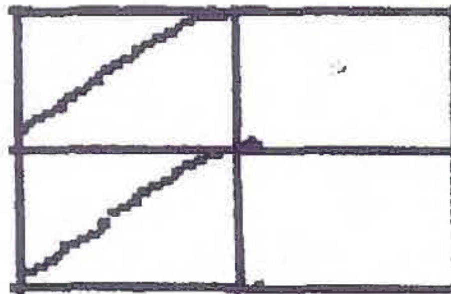
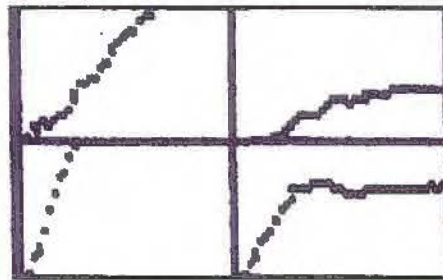


Fig. 52

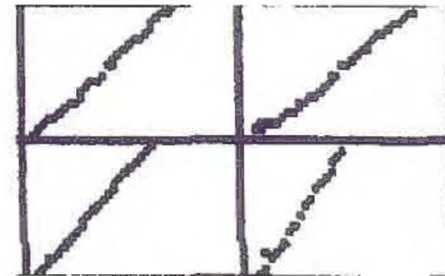
Individuals, heterozygous and homozygous for insensitive AChE, can be distinguished by biochemical assays



SS



RS



RR

Fig. 53

Mean frequency (both actual and predicted), of homozygous carbamate resistant *H. armigera*, in cotton populations (1988/99)

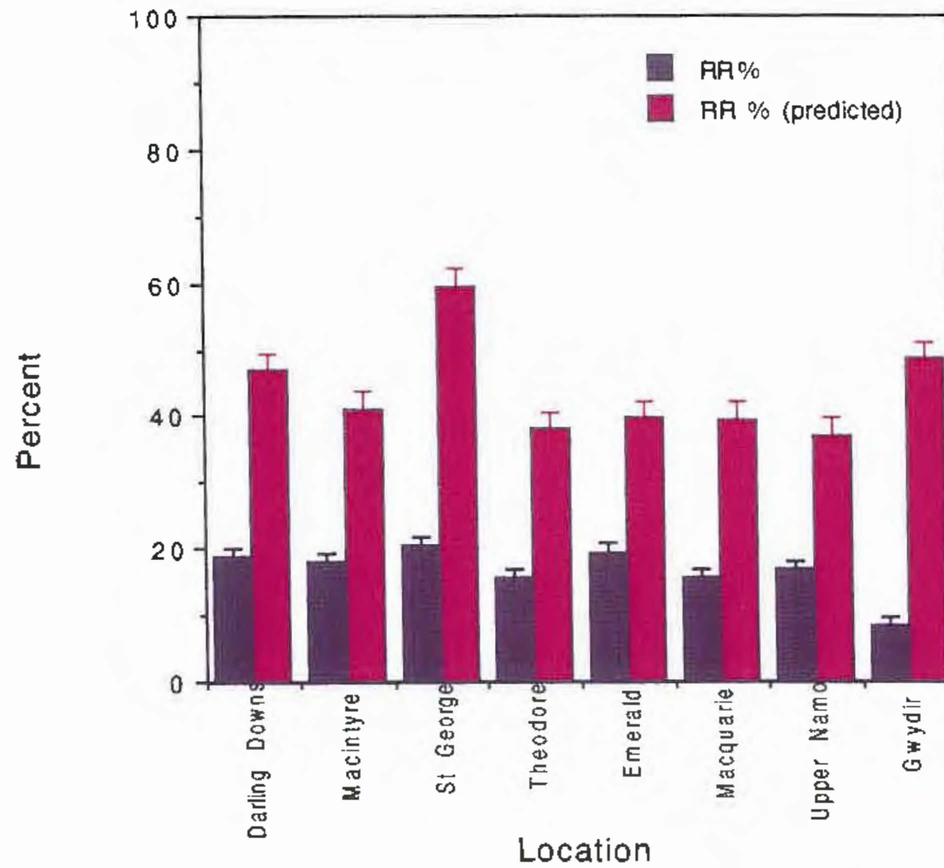


Fig. 54

Methomyl resistant *H. punctigera* have insensitive acetylcholinesterase

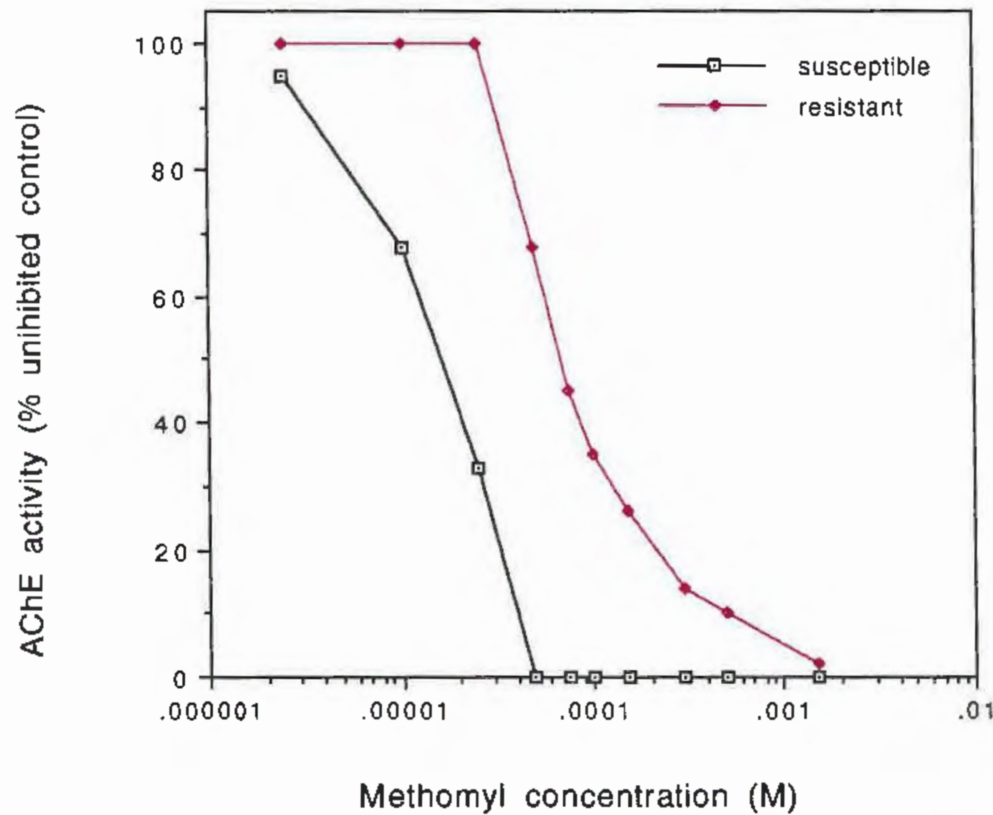
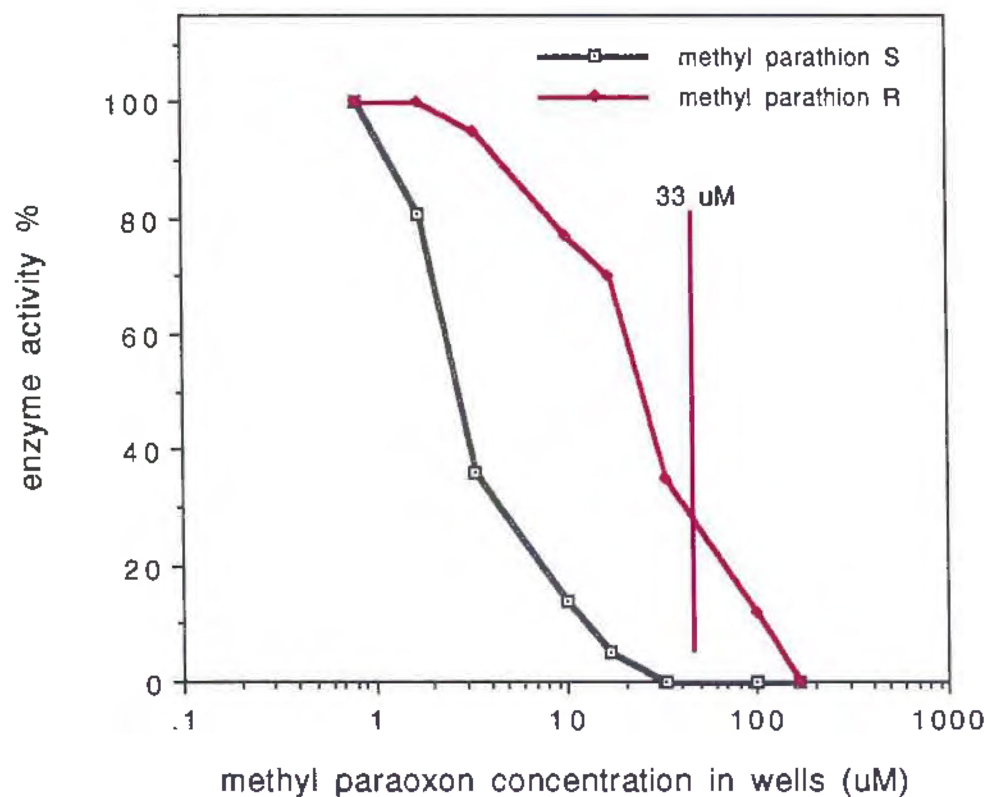


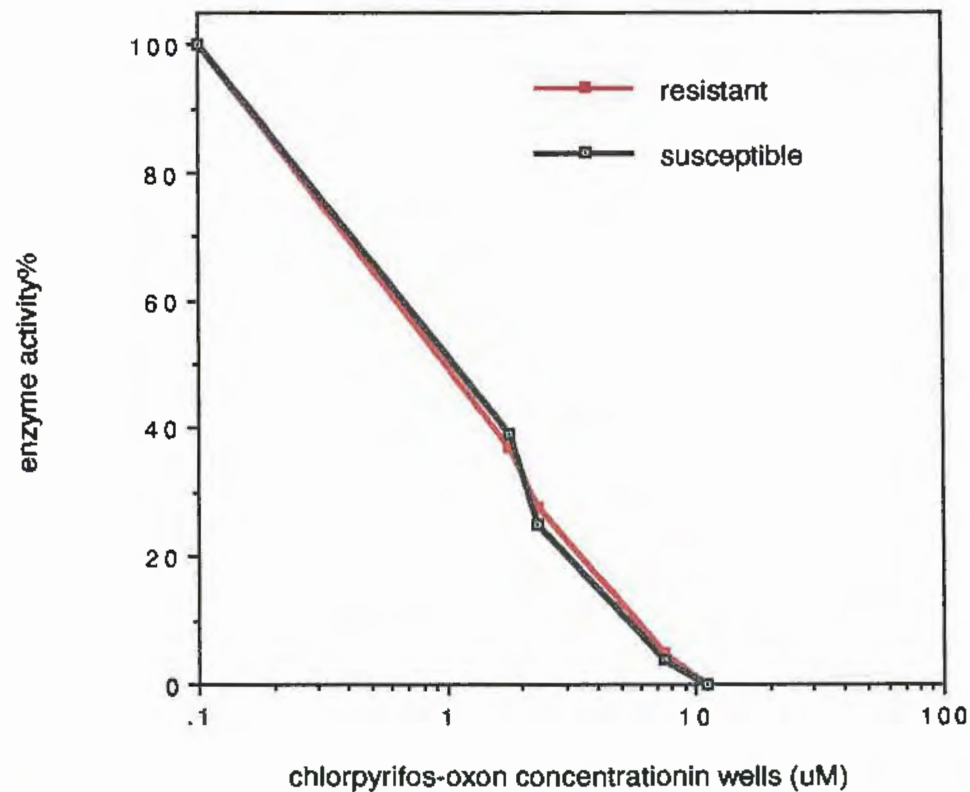
Fig. 55

Insensitive acetylcholinesterase resistance mechanism in profenofos/methyl parathion resistant *H. armigera*



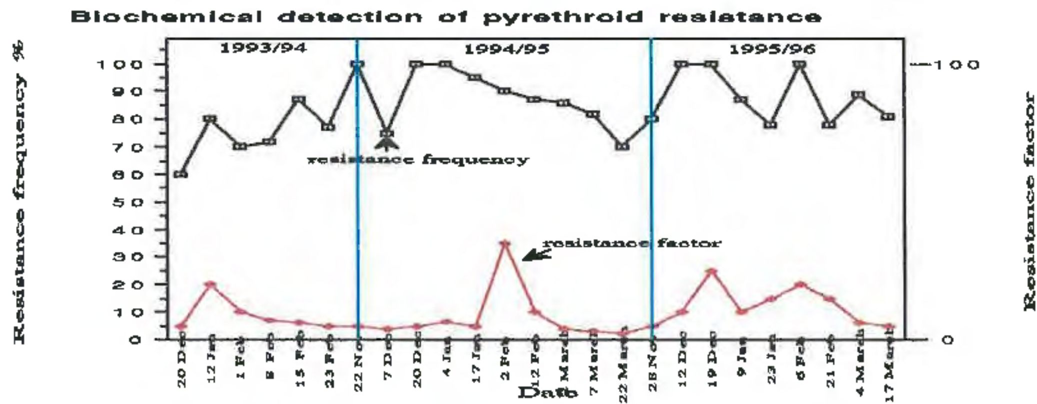
56.

Insensitive acetylcholinestase in profenofos and methyl parathion resistant *H. armigera*, is susceptible to chlorpyrifos.



57.

Pyrethroid resistance detection in Macquarie Valley, a comparison of biochemical assays and conventional bioassays in *H. armigera*



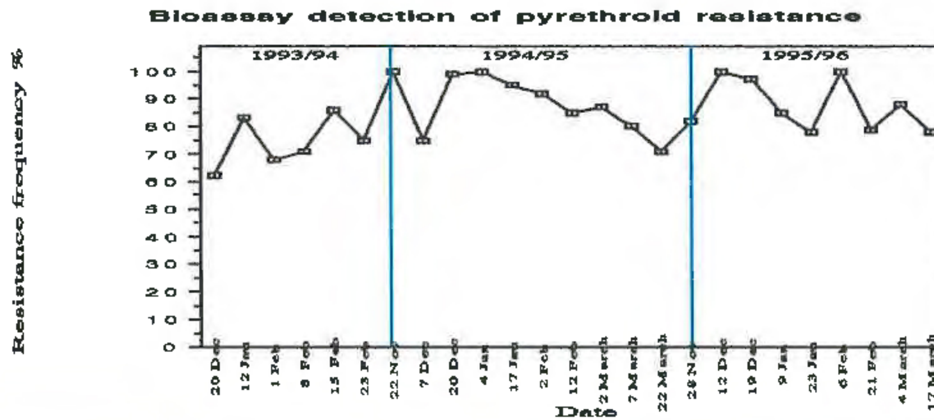
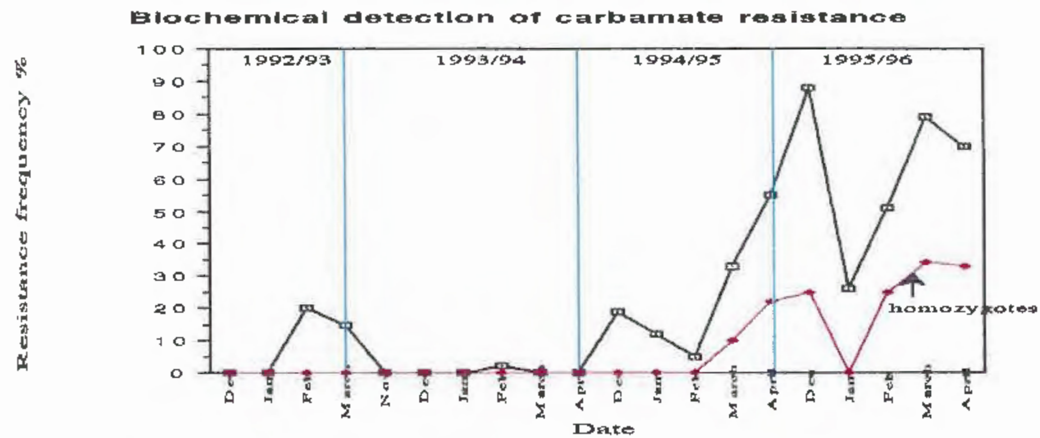


Fig. 58

Carbamate resistance detection in NSW and Qld., a comparison of biochemical assays and conventional bioassays in *H. armigera*



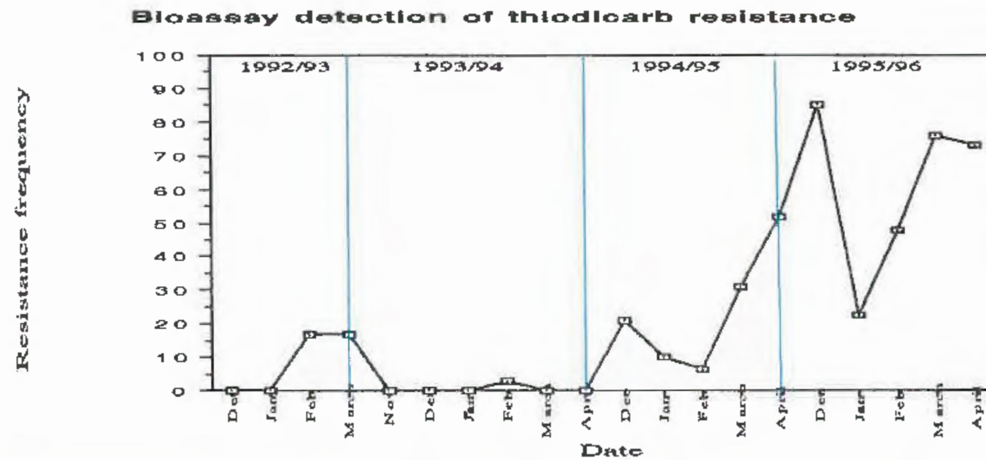


Fig. 59

Pyrethroid resistance detection kit

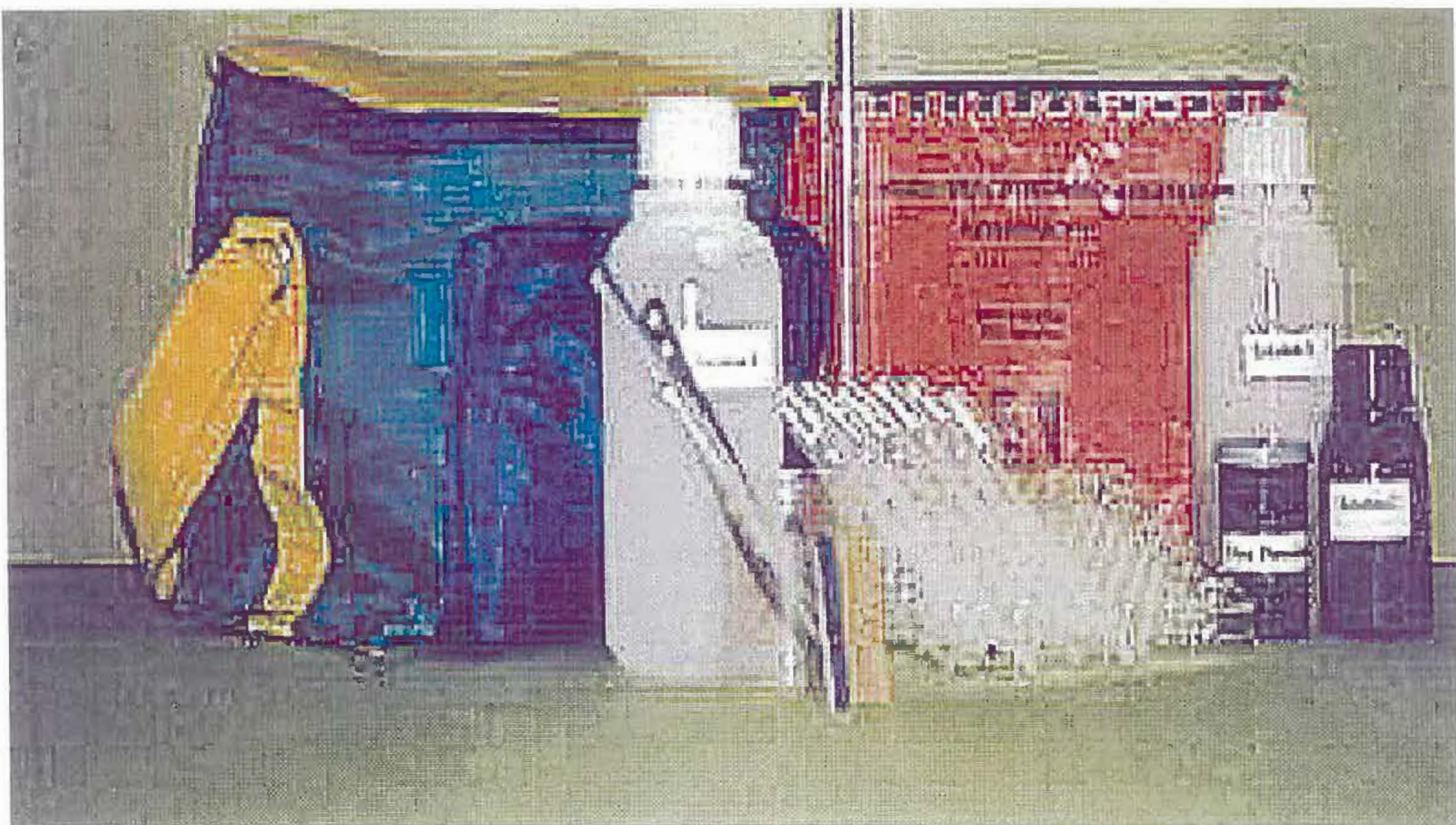


Fig. 60

Carbamate resistance detection kit

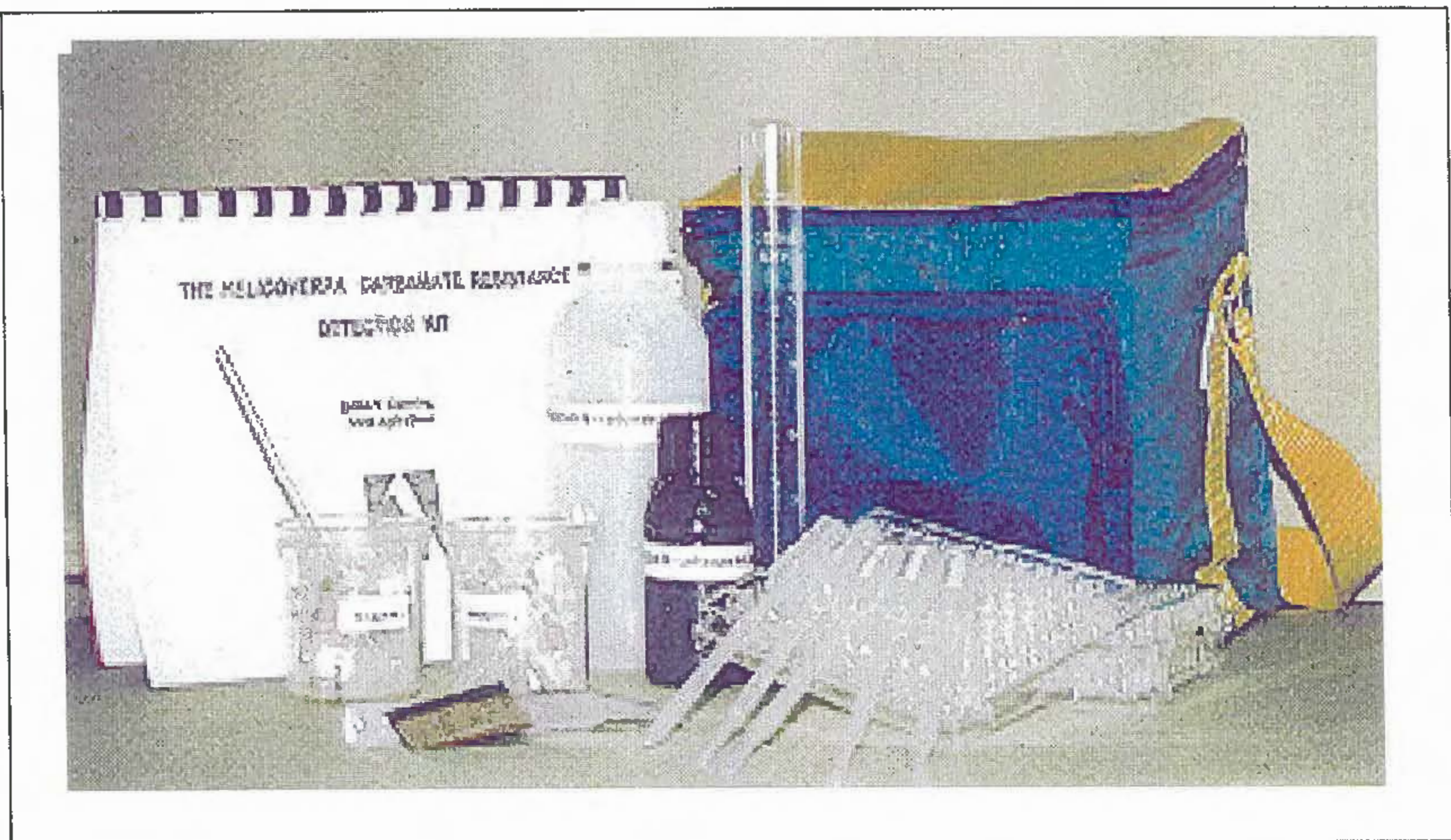


Fig. 61

Contents of resistance detection kit

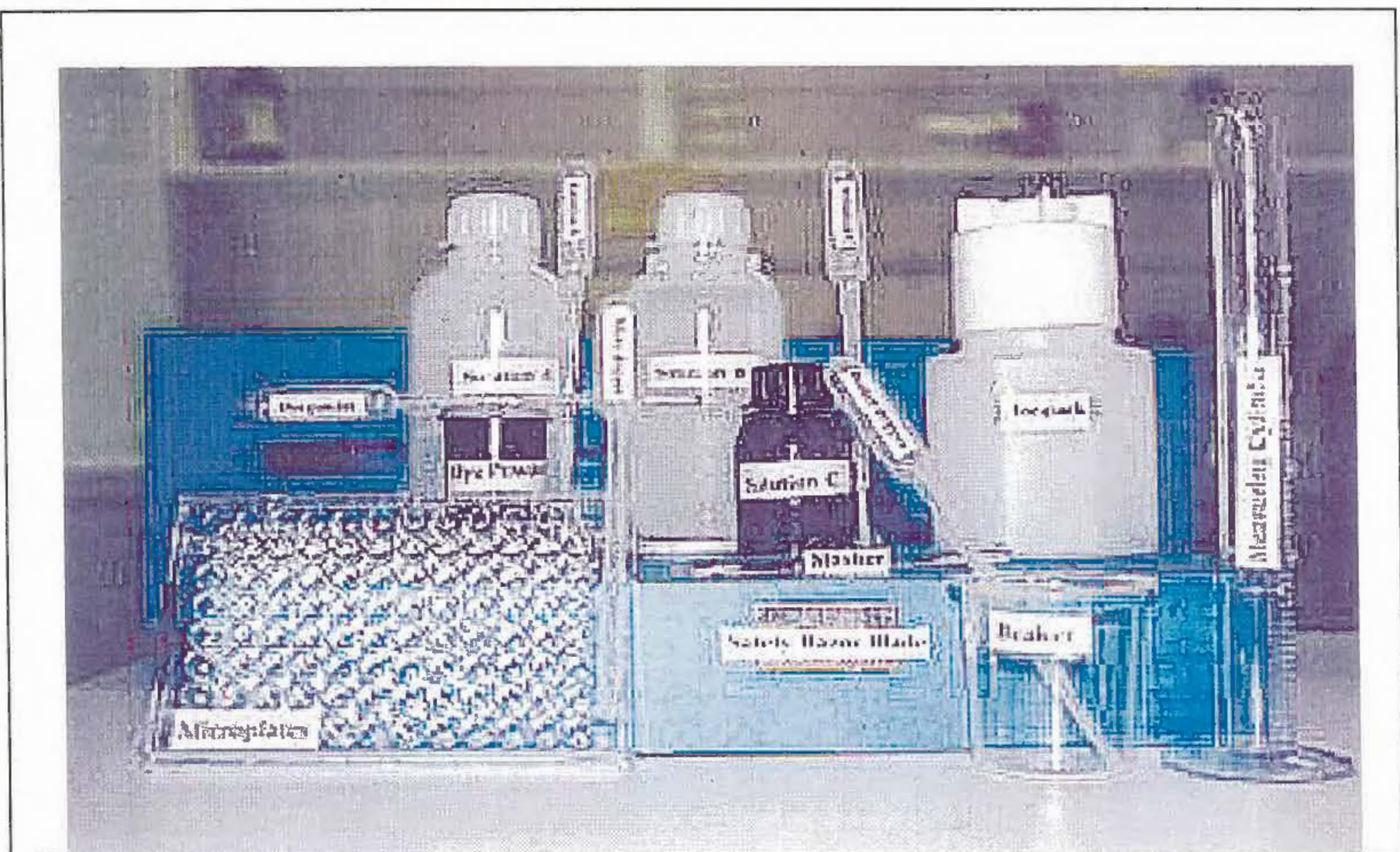


Fig. 62

Pyrethroid resistance detection

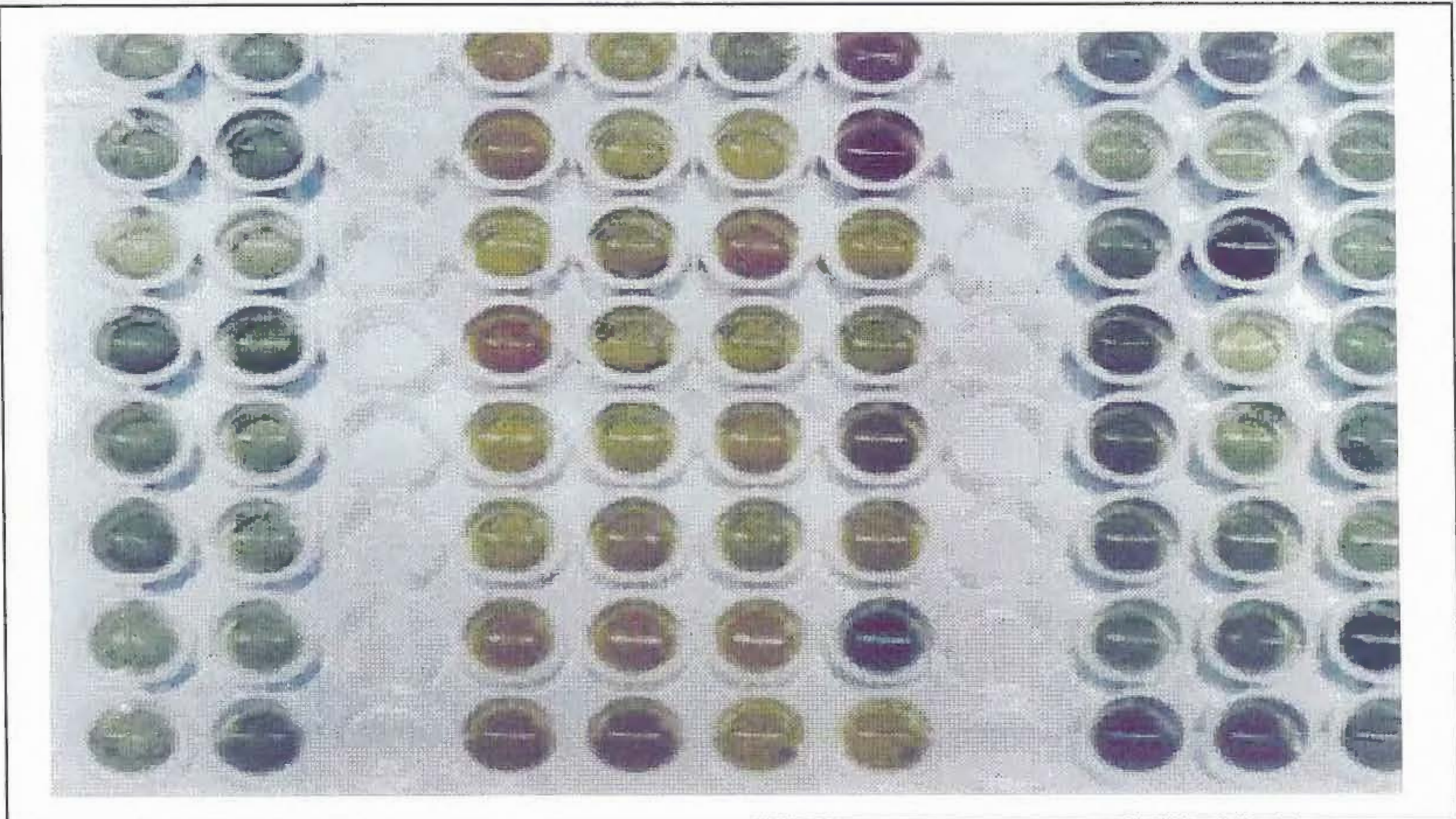


Fig. 63

Biochemical resistance detection kits used in the field

Fig. 64

Chlorfenapyr binds to esterase isoenzymes in resistant *H. armigera*

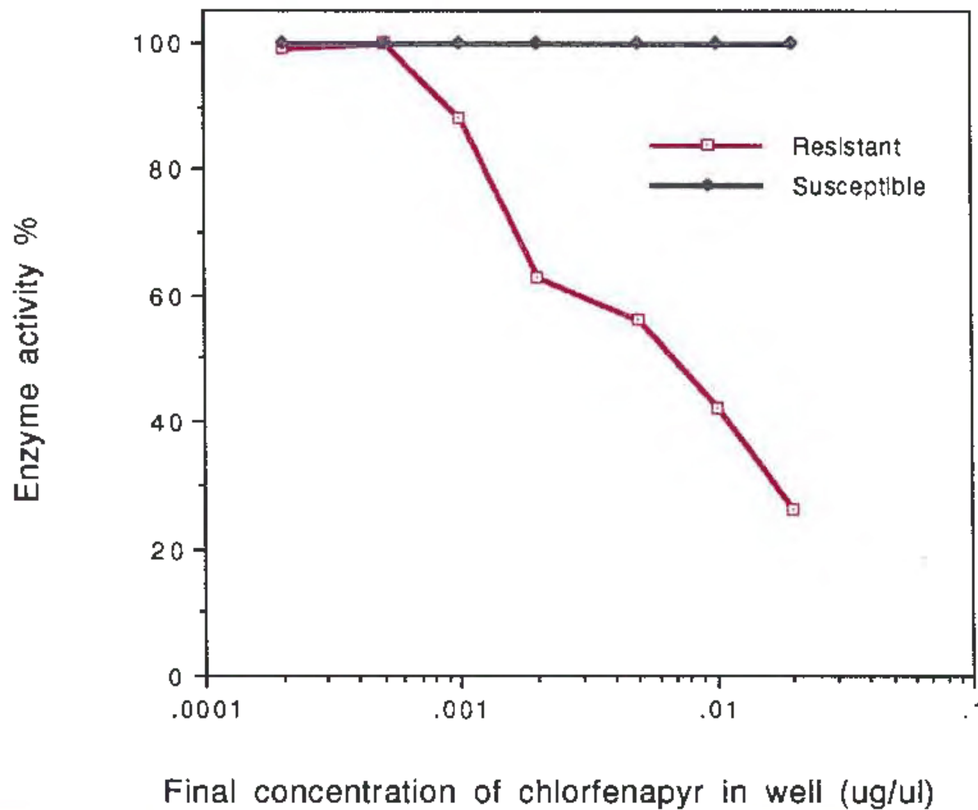
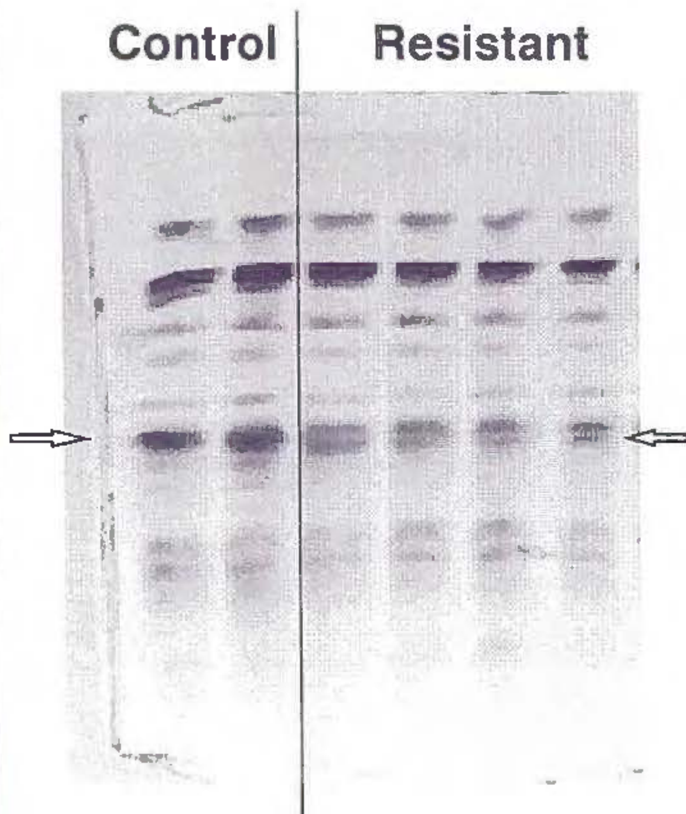


Fig. 65

Binding of piperonyl butoxide to esterases in pyrethroid resistant *H. armigera*



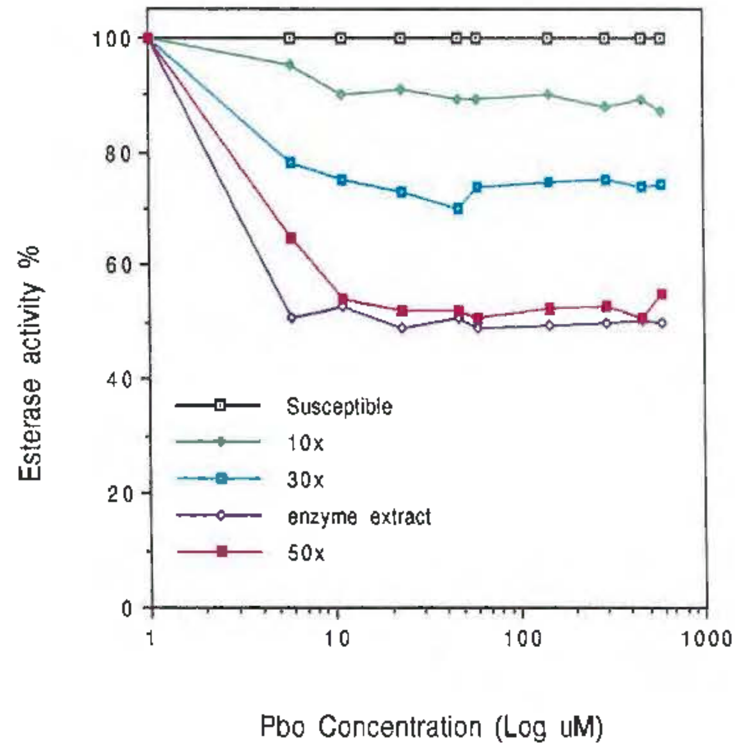


Fig. 66

Binding of propargite to esterase isoenzymes in pyrethroid resistant (35x) *H. armigera*

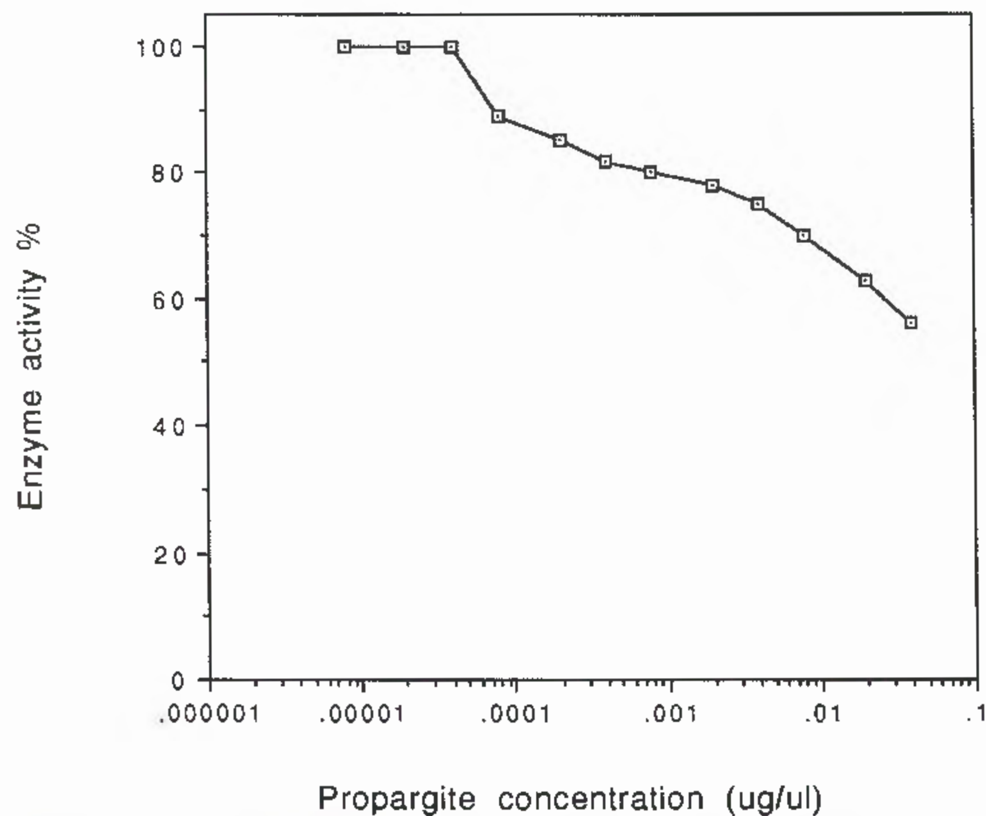


Fig. 67

Inhibitory effects of organophosphates (A acephate, B profenofos, C chlorpyrifos, E ethi on), on esterases in pyrethroid resistant *H. armigera*

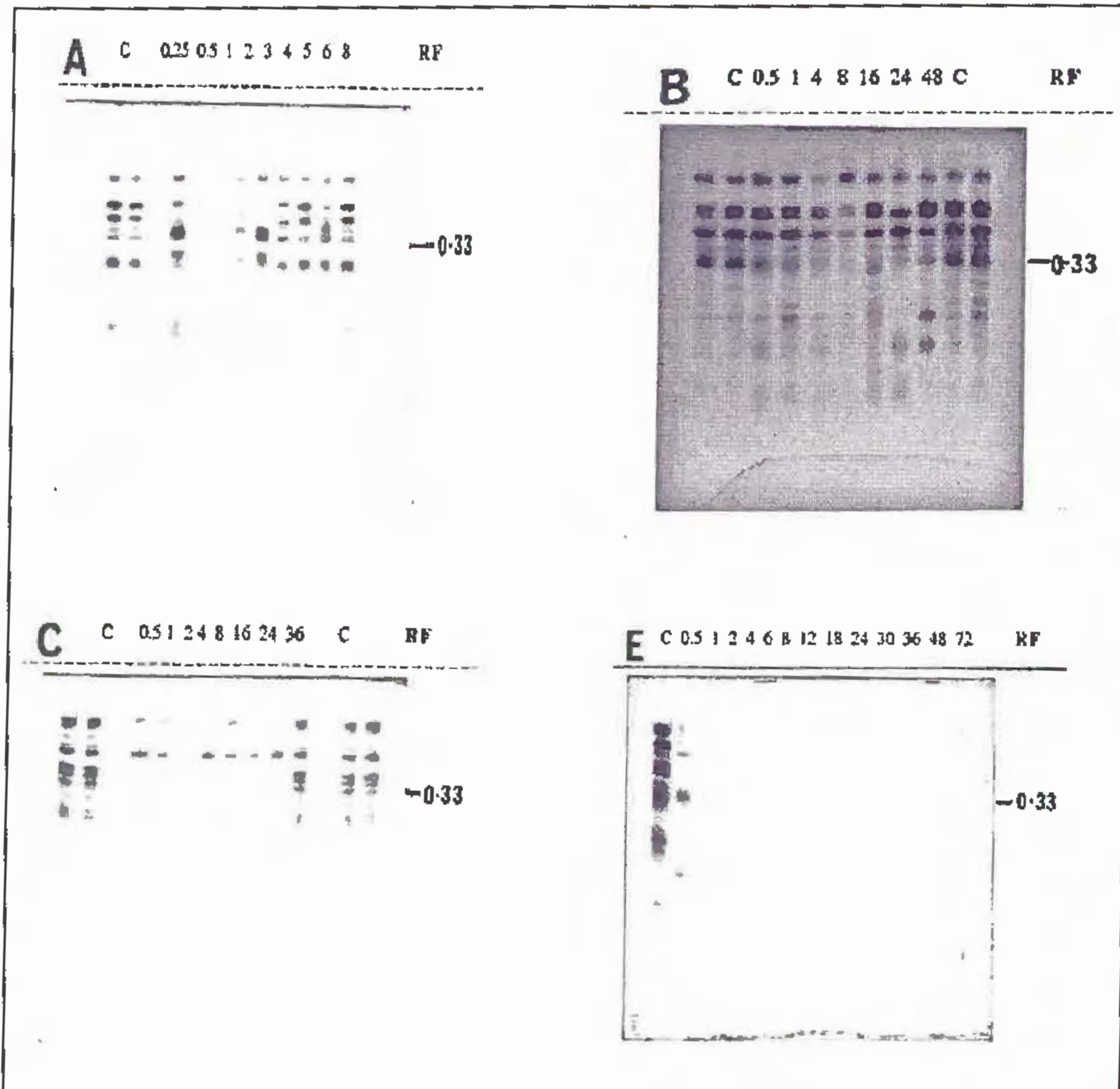
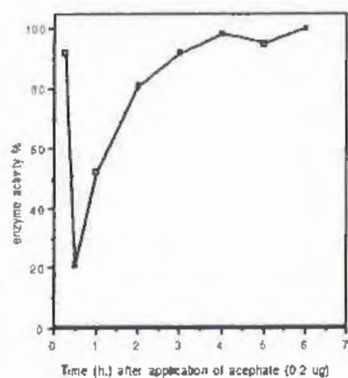
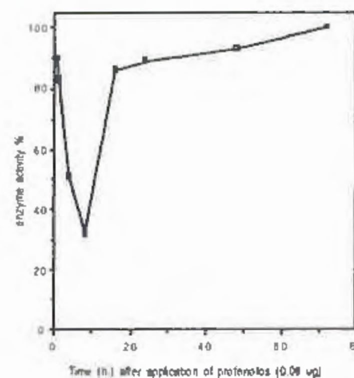
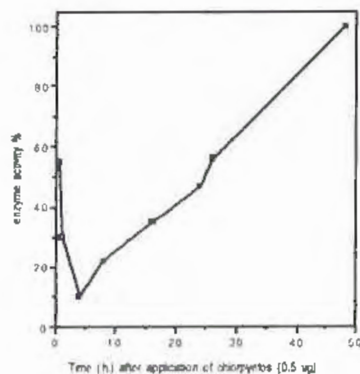


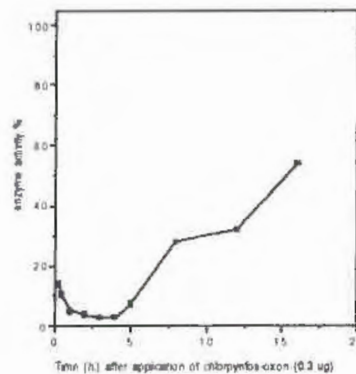
Fig. 68

Esterase inhibition by organophosphates in pyrethroid resistant *H. armigera*









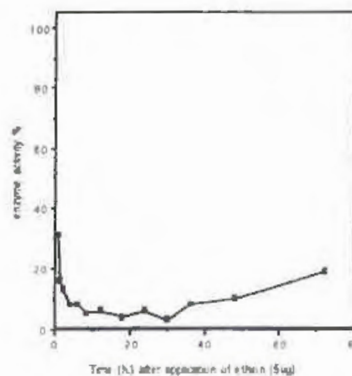
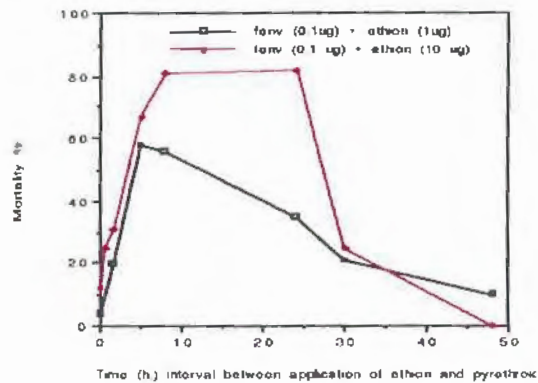
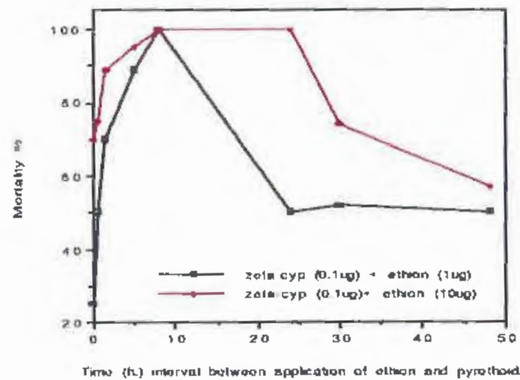


Fig. 69

Synergism of pyrethroids (fenvalerate and z-cypermethrin), by ethion





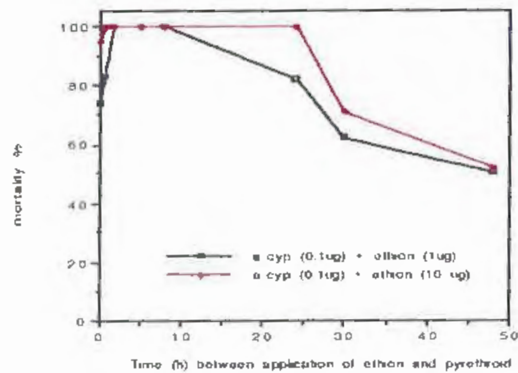
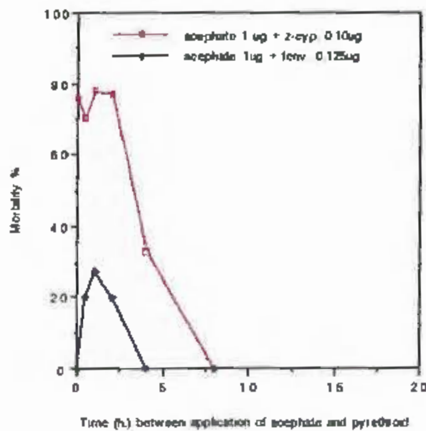
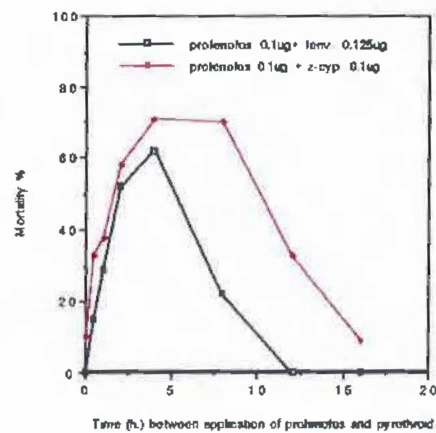
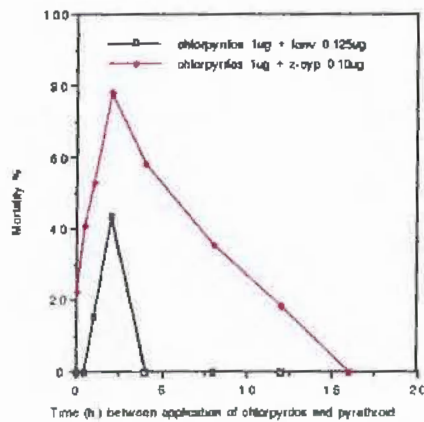


Fig. 70

Synergism of pyrethroids (fenvalerate and z-cypermethrin), by organophosphates







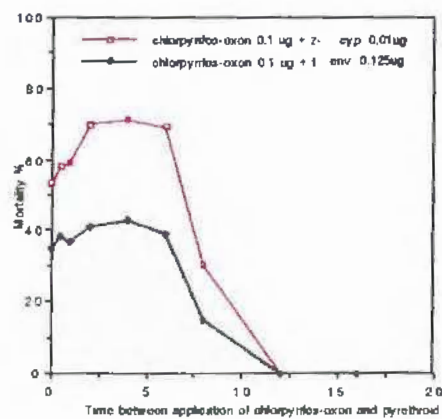


Fig. 71

Different toxicities of pyrethroids toward pyrethroid resistant *H. armigera* larvae

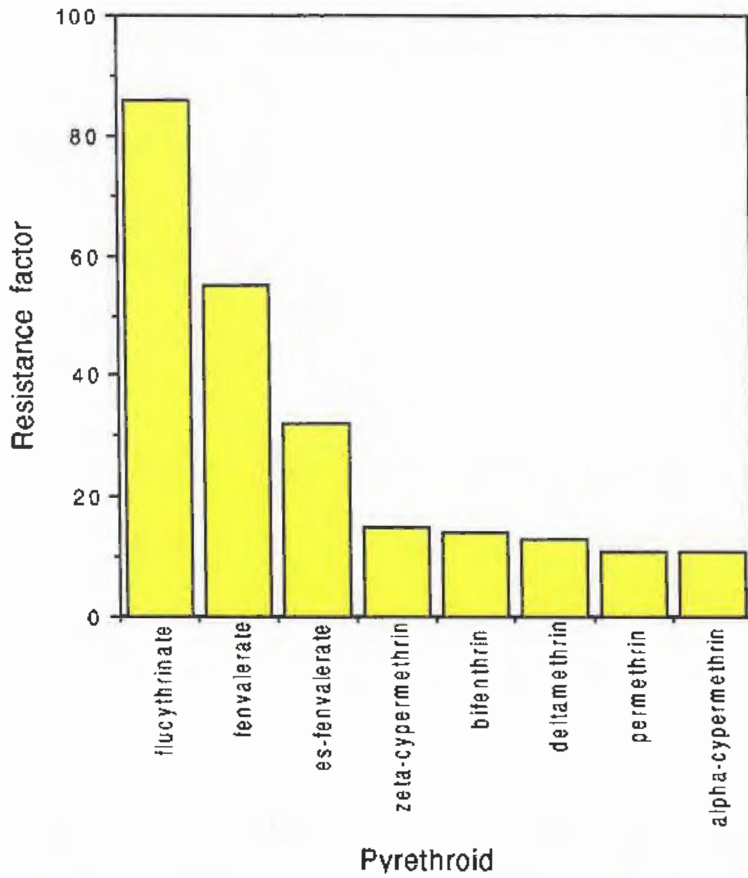


Fig. 72

Differential ability of pyrethroids to bind to esterases in resistant *H. armigera*

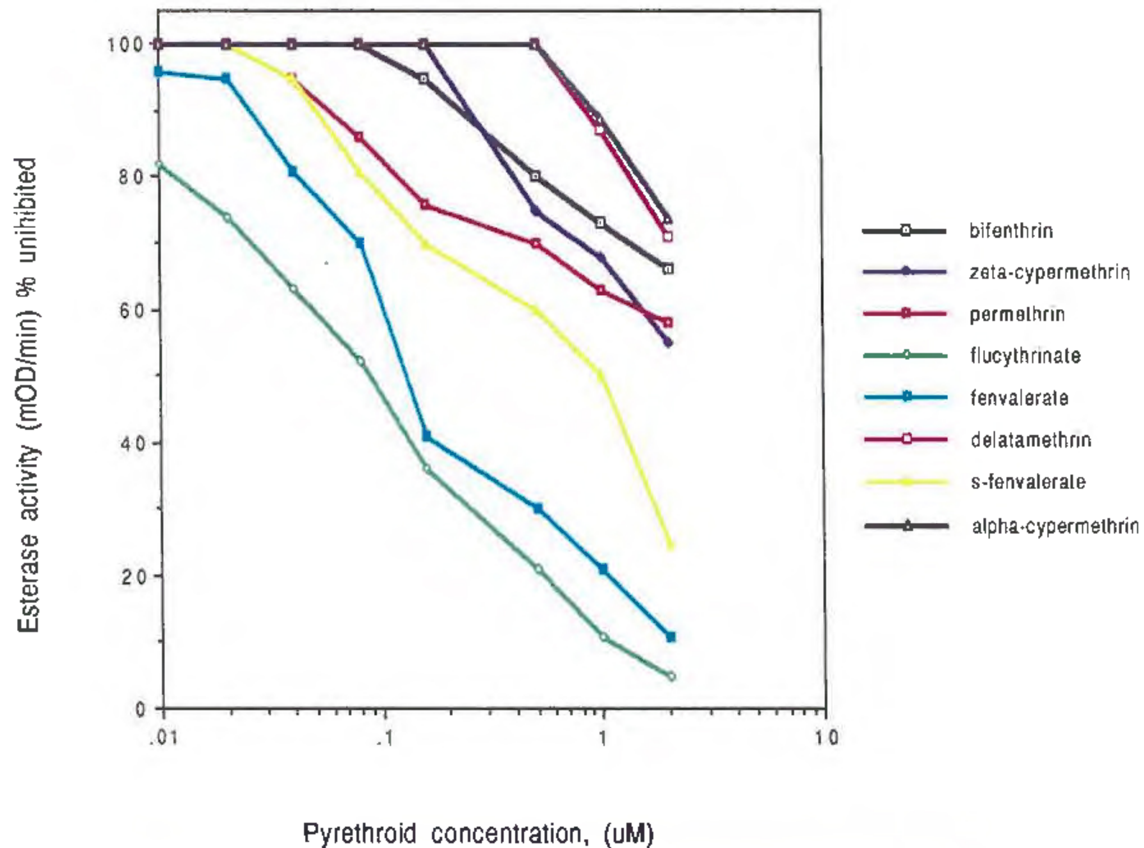


Fig. 73

Different ability of increasing amounts of fenvalerate and z-cypermethrin to bind to esterase isoenzymes in pyrethroid resistant *H. armigera*

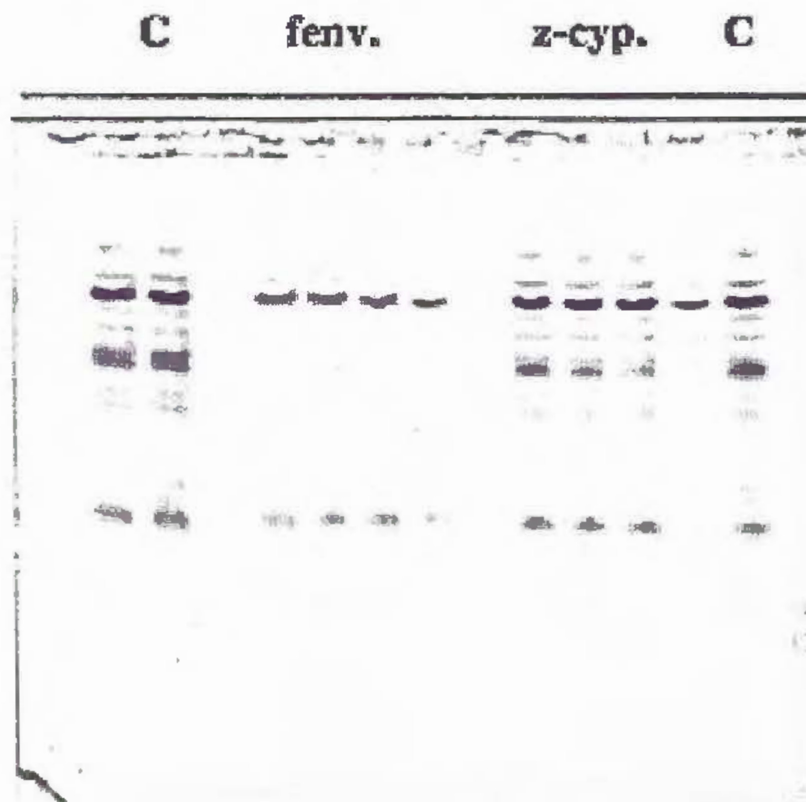
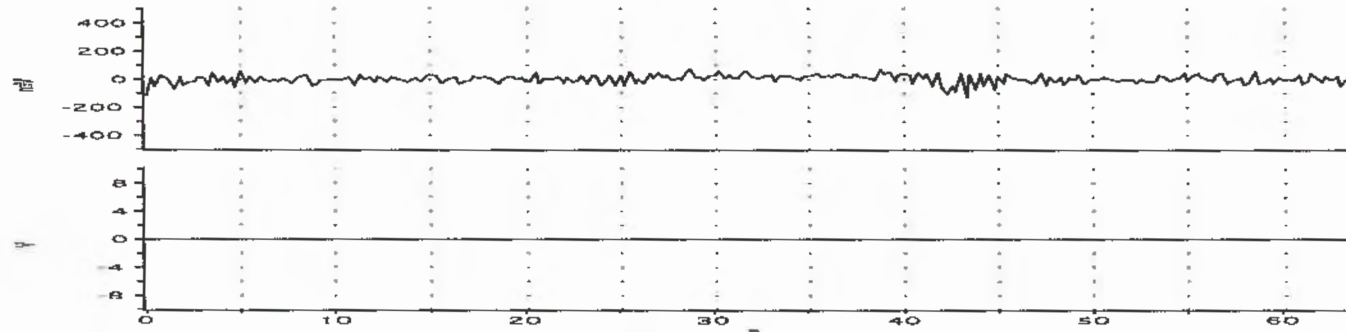


Fig. 74

Effects of bifenthrin on peripheral nerves in bifenthrin susceptible *H. armigera* larvae

Untreated control



Bifenthrin treated

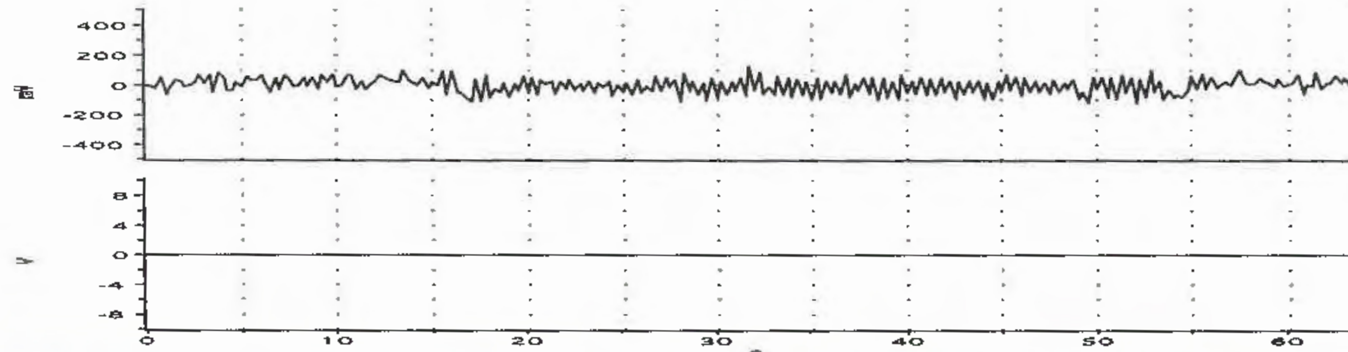


Fig. 75

Effects of bifenthrin on peripheral nerves in bifenthrin resistant *H. armigera* larvae

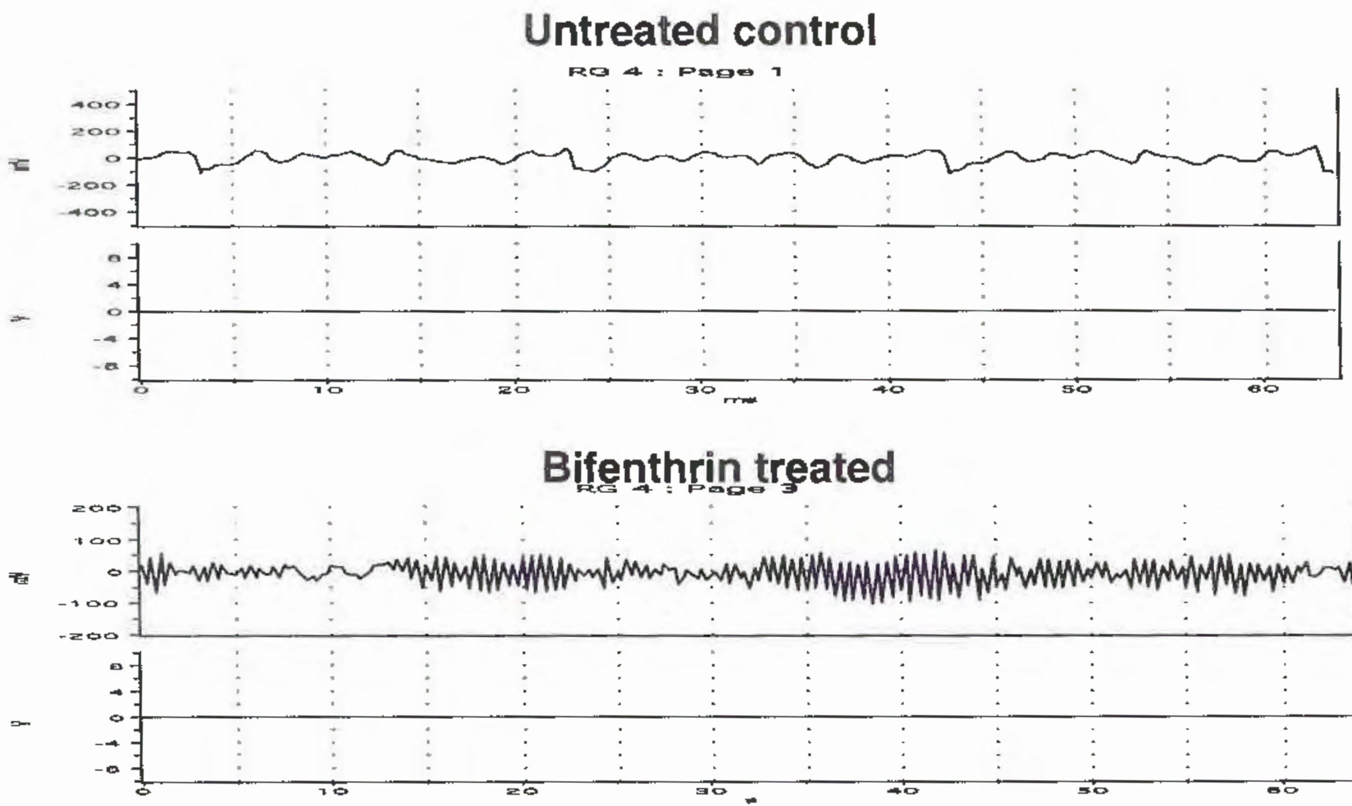


Fig. 76

Binding of bifenthrin to esterase isoenzymes in bifenthrin-resistant and -susceptible *H. armigera*

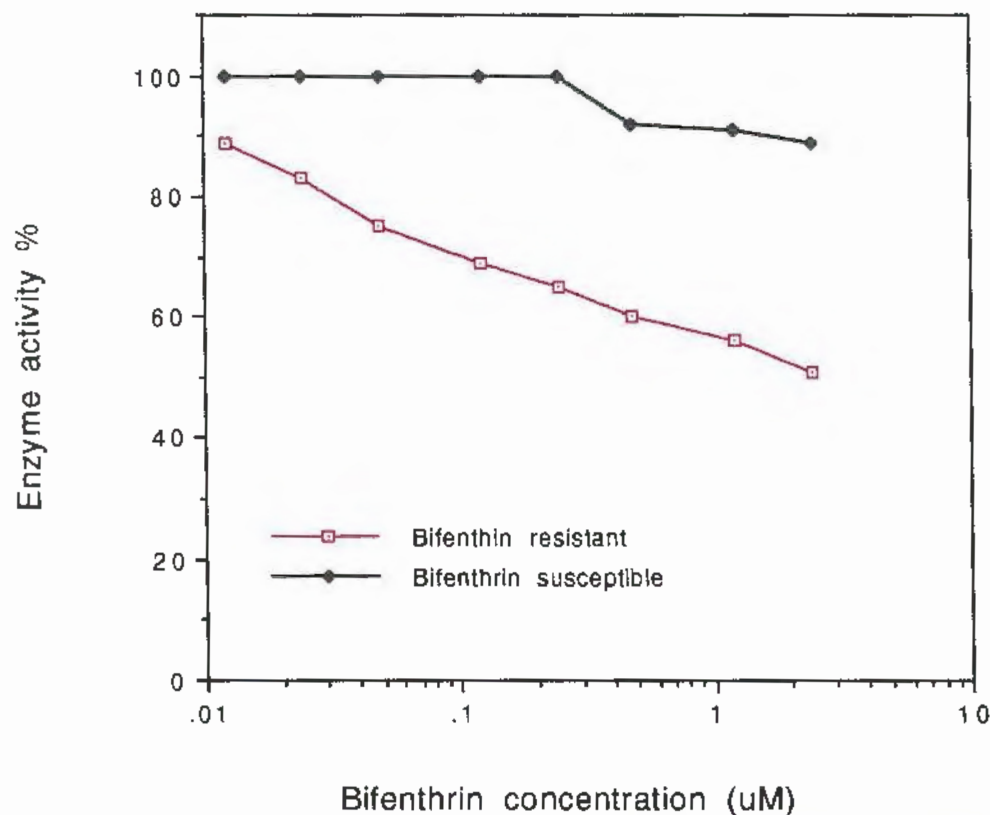


Fig. 77

Bifenthrin binds to particular esterase isoenzymes in bifenthrin resistant *H. armigera*

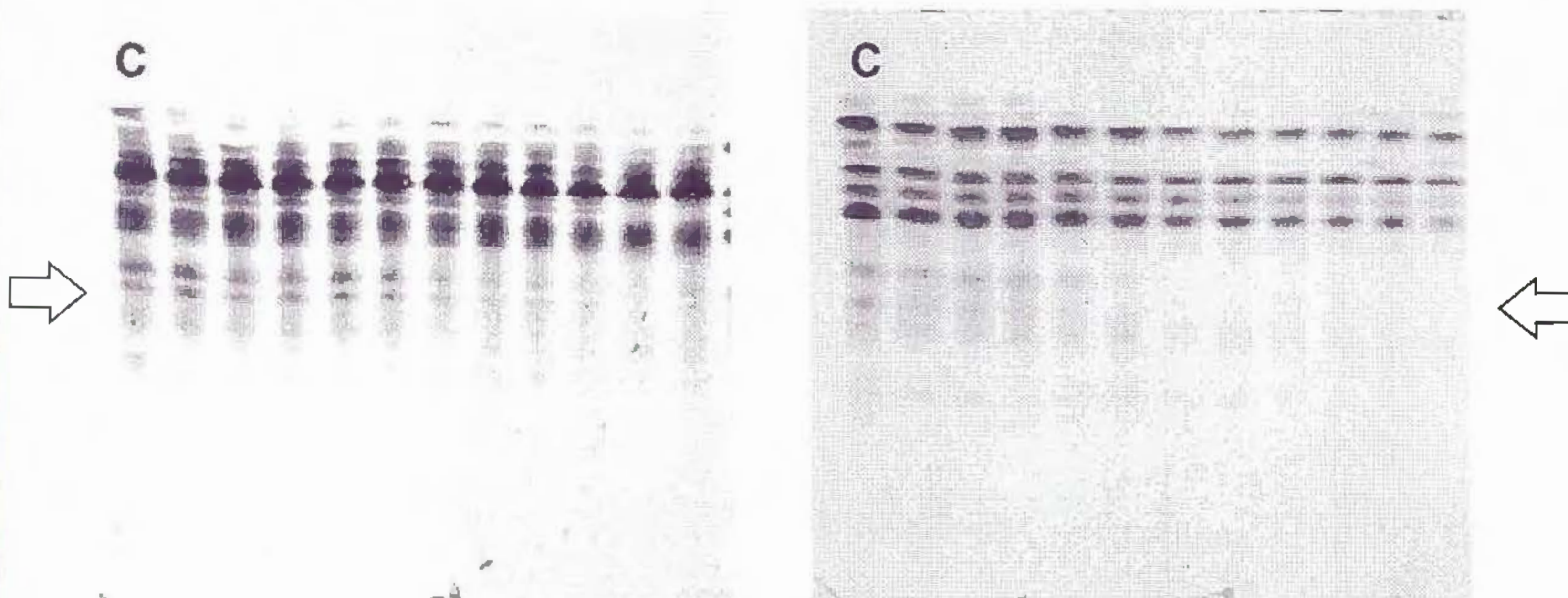


Fig. 78

Fenvalerate resistant *H. punctigera* have additional esterase isoenzymes that are not found in susceptible individuals

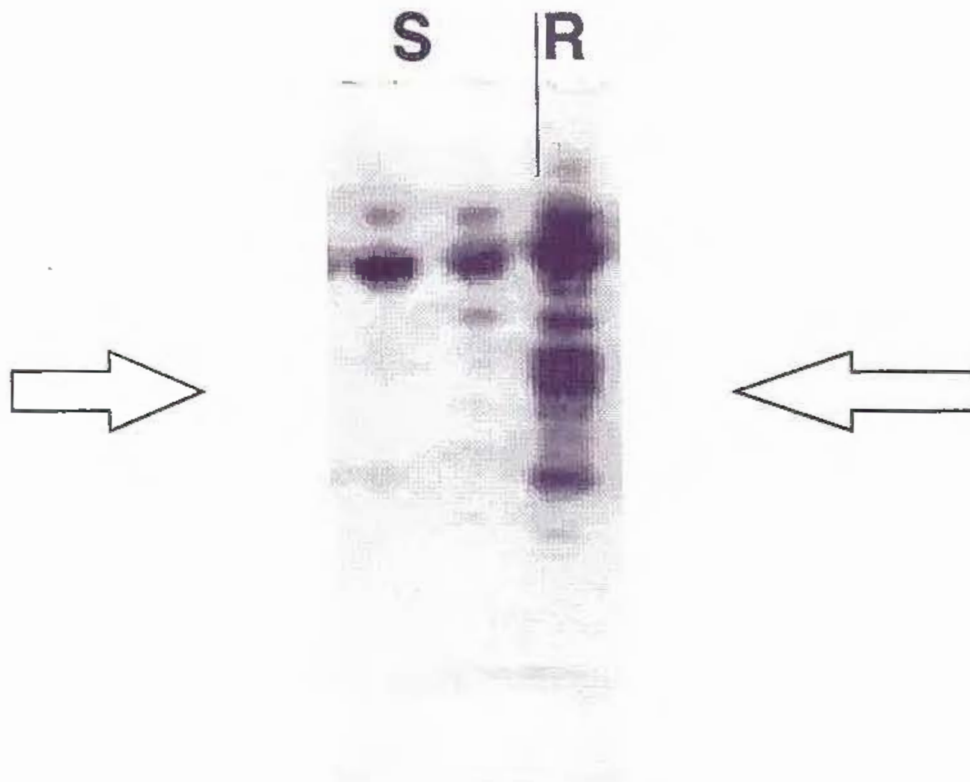


Fig. 79

Fenvalerate binds to esterase isoenzymes in resistant *H. punctigera*